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Proceedings
Symposium on Factors Producing Embryonic and Fetal Abnormalities, Death, and Abortion in Swine

Held at

Chicago, Ill.
October 2-3, 1967

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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Sponsored by:

The National Academy of Sciences

United States Livestock Sanitary Association

Agricultural Research Service, U.S. Department of Agriculture

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PREFACE

A Symposium on Factors Producing Embryonic and Fetal Abnormalities, Death, and Abortion in Swine was held in Chicago, Ill., on October 2-3, 1967.

At this symposium noted authorities and research scientists presented their findings as related to the intrauterine environment in swine. Their papers and the discussions that followed are included in this publication.

The symposium was sponsored by the National Academy of Sciences, United States Livestock Sanitary Association, and Agricultural Research Service, U.S. Department of Agriculture.

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FIRST SESSION: C.K. Whitehair,[1] Chairman

I. WELCOME AND PURPOSE OF THE SYMPOSIUM

By M. R. Clarkson[2] and D. P. Gustafson[3]

Dr. Clarkson:

Dr. Whitehair was kind enough to say that I don't need too much of an introduction. In looking around the audience this morning, I notice there are a great many of you who are not old enough to know who I am. This is kind of disconcerting. I look around and don't see too many I know. I am especially delighted to run across Dr. Kernkamp to exchange gossip with him.

I do want to give you a brief word of welcome to Chicago and to compliment the National Academy of Sciences, the Agricultural Research Service, and the United States Livestock Sanitary Association for organizing this symposium. The symposium is on a most important subject. A subject which I am sure you will give careful thought and consideration. They say a symposium is an excellent opportunity for individuals to get away from home, and thus to avoid the daily drudgery of work and the loneliness of thought. Well, I hope that doesn't occur here. This is scheduled for a work meeting and as a meeting from which we hope to get some thoughtful answers to the problems in this area.

I suppose, as everyone does in getting ready for a presentation, you look back on whatever information is available on the losses from embryonic and fetal abnormalities. The information in this area is conspicuous by its absence from most records and materials. I naturally turned to USDA figures in the latest report and totaled up the figures. It came to something like 7 percent losses from all causes of diseases in cattle and swine but embryonic and fetal losses are obviously not included. They are not included for a very good reason in that they are hard to identify and even harder to measure in percentage points or in dollar value. I don't know what your thoughts are in trying to put numbers on these losses, but as sort of a key to advancement it must be something like 25 percent of the productive capacity of our national swine herd. If this is so, and if it is so that we are going to need the tremendous increases in food production that normal increases in our population will bring about, we certainly cannot sit idly by and allow losses of this kind to continue without check.

I hope that the results of this conference will give us a benchmark for the future in terms of an assessment of these losses. Hopefully from the standpoint of someone such as I, this assessment may be translatable into figures, for it is only when we get down to figures that we can make the extent of these losses and their significance understandable to others. By others, I mean those who have influence over the allotment of funds for research and for programs that may be useful in bringing about alleviation of these losses.

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[1] College of Veterinary Medicine, Michigan State University, East Lansing: Chairman--Subcommittee on Prenatal and Postnatal Losses in Swine, Committee on Animal Health, National Academy of Sciences.

[2] Executive Secretary, American Veterinary Medical Association, Chicago, Illinois: Chairman, Committee on Animal Health, National Academy of Sciences.

[3] School of Veterinary Medicine, Purdue University, Lafayette, Ind., and Chairman, Committee on Transmissible Disease of Swine, U.S. Livestock Sanitary Association.

This stands to illustrate just once again the need in this country for a meaningful system of morbidity and mortality reporting of animals and animal losses from diseases of all kinds. The Animal Health Committee of the Academy is working in an effort to get such a system under way. We hope that we may see some provisions of these efforts in the next few years. This particular subject has been one that has attracted the attention of veterinarians for at least 75 years that I know about. Not all of it is from personal knowledge, but most of it is. I hope we can move along in that area. But, if we are able to use the results of this symposium as a benchmark, then we will have someting to go on. We can plan future research, and eventually we will practice the application of our new knowledge to the alleviation of the losses.

Finally, I would like to say to you that while you are here, the American Veterinary Medical Association (AVMA) offices are just down the street at 600 South Michigan. If there is any service we can be to you while you are here--if you have time to stop by--we will be very glad to see you. With this, I'll again bid you welcome to Chicago. Best of success in this important symposium.

Dr. Gustafson:

I would like to review for you something about the genesis of this particular meeting as we have thought of it. It would be, as Dr. Clarkson said, a work meeting, and one in which informality would be the dominant feature of the exchanges that are to be made. We had thought of a symposium or a meeting that was composed of those individuals who are actually involved in research and regulatory activities, people who were interested in the perinatal problems in swine. We found that on examination this was a little too much for the amount of time we would have. We wouldn't be able to provide the depth of consideration if we had it that broad. So, at the suggestion of one of our members, we confined it to combining our activities and the principal part of our interest to the intrauterine environment. However, we recognize that these things overlap and probably a considerable part of our discussion will have to face the extrauterine life of the baby pig, even though the underlying thought will be taken up with the intrauterine environment.

Now we had, as I say, thought about this being dominated by an informal atmosphere. Currently, all of our distinguished participants are not with us at the speaker's table--they are seated in the audience so that they may see that which will be projected on the screen and will be able to enter into the discussion.

In the last stage of the program format you can see that we have made an effort to provide a time for exchange between the participants and also persons in the audience.

I think you will find that as the meeting progresses there will be some deviation from the program, which was printed somewhat early. We hope this meeting will provide a cross-pollination between certain groups of scientists. We have animal scientists here to provide us with some of the physiological changes and activities that bear on the intrauterine environment, as well as those of us who are principally involved in experimental pathology or who are interested in infectious agents as they affect the baby pig.

We hope that exchanges between those of you who are interested in infectious processes and those interested in physiological environments will provide an opportunity to learn different approaches and methodology.

As this meeting goes on, I hope that we will feel free to make the most of it and to go home with a stimulated feeling that it has been very much worthwhile. Then our ends will be accomplished in that regard.

DISCUSSION

Dr. Whitehair:

I probably should mention that Dr. Kernkamp has been active in this field many, many years. We are happy he consented to come down here and guide us and give us help. We always appreciate his comments on many of these problems.

I think we are about to get into some of the fundamentals, some of the things we are here for. As Dr. Gustafson has pointed out, we probably don't know all the answers on many of these problems; but we are hopeful that many of you will contribute your thoughts and experiences and anything else that you might have. We would like to speed up our knowledge on many of these problems.

It is most appropriate that our first speaker, Dr. Conrad, will discuss the environmental factors influencing the pregnant sow. Dr. Conrad, a native Hoosier, was trained primarily in the area of nutritional biochemistry with his bachelor's, master's, and doctor's degrees in this area. He has been long active at Purdue in some of these practical problems under field conditions. I like to think that Joe has not only been familiar with problems in Indiana, but more or less in some of the worldwide problems, having spent some time in a foreign assignment. So, we are very happy that Dr. Conrad will kick off our meeting this morning. He will tell us some of the experimental factors that influence the pregnant sow.

II. SCIENTIFIC PRESENTATIONS AND DISCUSSIONS

Paper No. 1

ENVIRONMENTAL FACTORS INFLUENCING THE PREGNANT SOW

By J. H. Conrad[1]

Environment may be defined as the surrounding conditions, influences, or forces that influences, or forces that influence or modify. Stated more specifically, it is the whole complex of climatic, edaphic, and biotic factors that act upon an organism or an ecological community and ultimately determine its form and survival.[2] Environmental factors could include a wide variety of subject matter areas. However, since several of these areas will be covered in subsequent papers at this symposium this paper will treat those environmental factors not covered by assigned topics in the succeeding presentations. This, therefore, will include two areas that may be designated thermal and nonthermal.

Most of the environmental research involving the porcine species has dealt with temperature effects on performance. Under certain laboratory conditions, temperature and humidity have been rigidly controlled. But, in many investigations, performance and behavior have been evaluated in response to quasi-controlled conditions made possible by modification of facilities and equipment. Reviews on the physiological mechanisms of adaptation and the responses of animals to climate and temperature include reports by Andrews (3) and Bond (5). An excellent comprehensive review of the experimental data available on environment and facilities in swine production is that of Jensen (12).

Environmental temperature control is of major consideration with the porcine. Swine are homeotherms, yet they are nonperspiring and have a relatively inefficient thermo-regulatory system. Most of the body heat is dissipated by evaporation from the lungs. Radiation, conduction, and convection also aid in heat dissipation; but the extent of each is dependent upon temperature, humidity, and other factors of the environment.

Our immediate concern, therefore, is the effect of environmental temperature on the pregnant sow. The investigator has been concerned with determining how elevated environmental temperature affect ova fertilization, embryological mortality, and subsequent litter size.

Physiologists have reported that under uncontrolled environmental conditions at least 40 percent of all ova ovulated are lost before parturition (14). Of this 40 percent mortality, approximately 85 percent of the loss is incurred before the 25th day of pregnancy. Furthermore, prenatal mortality and degenerate fetuses at different stages of pregnancy have been reported in detail (15). These studies indicate that embryonic losses appear to increase markedly during the 60- to 69-day period which coincides with the period when the growth of the fetus overtakes the growth of the

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[2]Webster's Third New International Dictionary Unabridged, p. 760. 1961.

Table 1.--Prenatal mortality by 10-day intervals [15]

Stage of pregnancy (days)	Corpora lutea (av.)	Normal fetus (av.)	Percent loss embryos	Percent degeneration
20-29	12.8	7.5	41	0
30-39	18.2	13.8	25	9.2
40-49	15.8	9.6	39	4.3
50-59	17.0	10.2	40	4.2
60-69	17.9	11.3	37	17.7
70-79	16.6	10.8	35	7.8
80-89	16.0	12.0	25	8.9
90-99	16.4	9.0	46[1]	6.0
100-113	16.7	8.2	51[1]	7.3
Av.	16.5	10.0	40	7.7

[1] Significantly (P 0.01) greater than between 20 and 89 days.

fetal membrane. Significant prenatal mortality was also found to occur between the 90th and 112th day (table 1). This increased embryonic loss prior to parturition is thought to be due to overcrowding in the uterus. Consequently, these three periods--the first 25 days, the 60 to 69th days, and the last 21 days--appear to be critical during gestation when thermal stress may elicit a detrimental response.

Thermal Stress and Stage of Pregnancy

One of the first studies on the effects of thermal stress on pregnant sows was reported by Heitman and coworkers (10). Thirteen sows that were at least 85 days pregnant were continuously exposed to high temperatures, averaging 99° F. for periods of 8 days. In this study 99° temperatures produced pronounced evidence of physical discomfort, but no abnormalities occurred with regard to litter size or health of the 13 sows. One aborted from other causes, one died from heat prostration, and 11 farrowed normally.

A comparative study was conducted with an open sow and one 85 days pregnant. During the 8-day trial at 98° F. the open sow had a minimum respiratory rate of 64 per minute as compared to 186 for the pregnant sow. Even though the open sow lost 33 pounds in 8 days the results indicated she was not as stressed by high temperatures as the pregnant sow. This early study indicated that pregnant sows during the last 3 weeks of gestation have difficulty dissipating the additional heat load provided by thermal stress, yet the dam will die of heat prostration before embryonic mortality and subsequent abortion occurs.

Embryonic mortality and litter size as influenced by thermal stress before 25 days' post coitum have been the objectives of recent investigations. Previous research with species other than swine have indicated that the pregnant female is most sensitive to thermal stress very early in gestation. Dutt (6) and Dutt and others (7) reported a decrease in sensitivity of ewes to the adverse effects of elevated temperatures as pregnancy advances with relative resistance to heat-induced reproductive damage by the 8th day of gestation. Likewise, Alliston and Ulberg (2), using the technique of ovine ova transfer, reported that a 90° F. temperature was more detrimental during than after, the first 3 days following mating. Ewes exposed to elevated ambient temperatures up to the end of estrus had a greater embryonic mortality than ewes in which heat treatment was not applied until the end of estrus (Woody and Ulberg, 24). Laboratory animals have also been observed to exhibit a decrease in heat sensitivity with increasing age of the embryo (Fernandez-Cano, 9 Hsu, 11).

Three trials have recently been conducted at Purdue University (18) in which sows were placed in psychrometric chambers on day 1, 5, or 20 of gestation and were exposed to temperatures of 95° or 98° F. for 1, 2, or 5 days. In trial 1 (table 2) exposure of sows to 95° temperature for 24 hours on day 1, 5, or 20 of pregnancy resulted in 13.2 percent fewer, 7.4

Table 2.--Effects of exposure of pregnant sows to 95° F. for 24 hours (18)

Day of pregnancy	Average number of--			Percent of viable embryos
	Corpora lutea	Viable embryos	Dead embryos	
1	14.4	9.3	0.1	64
5	13.8	12.0	0.4	85
20	14.9	10.8	0.4	72
Control	14.5	11.3	0.0	78

Table 3.--Effects of exposure of pregnant sows to 95° F. for 48 hours (18)

Day of pregnancy	Average number of--			Percent of viable embryos
	Corpora lutea	Viable embryos	Dead embryos	
1 - Control	21.8	17.0	1.2	78
1 - Stressed	19.9	14.7	1.3	74
20 - Control	18.5	17.0	0.5	92
20 - Stressed	20.4	16.0	0.7	78

Table 4.--Effects of exposure of pregnant sows to 98° F. for 5 days (18)

Day of pregnancy	Average number of--			Percent of viable embryos
	Corpora lutea	Viable embryos	Dead embryos	
1 - Control	16.0	11.0	1.0	69
1 - Stressed	17.3	[1] 6.8	1.0	[1] 39
20 - Control	22.0	14.0	0.0	64
20 - Stressed	15.8	12.2	0.8	77

[1] Significantly ($P < .05$) smaller than control and 20-day stressed value.

percent more, and 5.3 percent fewer viable embryos per 100 corpora lutea, respectively, than controls. Increasing the duration of exposure of pregnant sows to 95° for 48 hours (trial 11 - table 3) on day 1 or day 20 of pregnancy, resulted in a 4.1 percent and 13.6 percent decrease, respectively, in the number of viable embryos per 100 corpora lutea as compared to controls maintained at 60°. However, none of the differences in the first two trials were significant.

In a third trial exposure of sows to 98° F. with a relative humidity of 50 percent for 5 days beginning on day 1 of pregnancy resulted in a 50 percent decrease in viable embryos per 100 corpora lutea as compared to no effect on the number of viable embryos for those stressed on day 20 of pregnancy (table 4). However, the combination 98° temperature and 50 percent relative humidity for 5 days was severe enough to kill eight of the 22 heat-stressed sows in trial 3. Average rectal temperatures reaches a high of 105° at which time the average respirations per minute were 185.

Similar studies were conducted at Ohio State University (17) in which a total of 240 sexually mature Duroc gilts were exposed to 80°, 86°, or 92° F. temperatures for one estrual cycle before breeding and for the first 25 days of gestation. With an increase in temperature there was a significant decrease in feed intake and an increase in rectal temperature. At 92° average daily gain and the number of corpora lutea present 25 days after breeding were also lowered (table 5). Breeding performance may have been adversely affected by the elevated environmental temperatures. Among the 60 gilts exposed to 92° eight returned to estrus after breeding, the same number as among the 80 gilts exposed to 86° in the second trial. At the two higher temperatures, five gilts that were cycling when put into the controlled temperature rooms failed to return to estrus during a 49-day period. Those affected in this manner were then removed to temperatures of 70° and consequently returned to estrus in from 4 to 17 days.

Table 5.--Effects of temperature on conception rate and litter size at 25 days

Item	80° F.	86° F.	92° F.
Settled at first service, percent	90.5	84.8	76.7
Failed to settle, percent	9.5	12.7	18.3
Estrus not detected, percent	0	2.5	5.0
Average corpora lutea, 25 days	14.2	13.6	13.2
Average live embroys, 25 days	10.3	9.7	9.6

Table 6.--Effects of exposure of gilts and boars to 60° or 90° F. (21)

Environmental temperatures, °F.		Average number of--		Percent of viable embryos
Gilts	Boars	Corpora lutea	Viable embryos	
60	60	14.9	11.2	75
60	90	14.3	12.1	85
90	90	14.3	10.8	76
90	60	12.9	10.0	78

Both gilts and boars were kept at 60° F. or 90° temperatures and used in reproductive studies at the Florida Agricultural Experiment Station (21). The four treatment groups included: (1) gilts kept at 60° and bred to boars kept at 60°, (2) gilts kept at 60° and bred to boars kept at 90° and (3) gilts kept at 90° and bred to boars kept at 90°, and (4) gilts kept at 90° and bred to boars kept at 60°. In these studies (table 6) gilts maintained at 60° had 14.6 corpora lutea compared to 13.6 corpora lutea for those at 90°. The average number of live embryos at 25 days was 11.7 at 60° and 10.4 at 90° in gilts bred to boars kept at the two temperatures. There were 0.9 more embryos in gilts bred to boars at 90° compared to gilts bred to boars at 60°, indicating the high temperature did not reduce fertility of the boars.

Some dramatic results from sprinkling sows during the last 60 days of gestation have been reported by investigators at the Oklahoma Agricultural Experiment Station (22). Sprinkling was begun on June 15th, and the animals were moved to the farrowing barn in August and September. The sows sprinkled during pregnancy farrowed 10.06 live pigs per litter, which was 2.35 more pigs per litter than those farrowed by the sows that had not been sprinkled. The difference was statistically significant. The sprinkled sows weaned 7.76 pigs per litter--2.05 more pigs per litter than their nonsprinkled mates. The difference was statistically highly significant. Although outstanding results were obtained in this study similar results haven't been reported by other investigators.

Effect of Season on Reproductive Performance

It has generally been accepted by swine producers that season affects productivity of the sow. Data in support of this were reported by Wallace and Combs (20) from research conducted at the Florida Station. A summary of 2 years' data involving 224 litters and 2,211 pigs showed a consistent advantage in concpetion rate, number of pigs weaned per litter, and weaning weights when breeding occurred during the cooler months (table 7). Management and nutritional factors were similar during all farrowings.

Two other studies involving different geographic areas have failed to produce results indicating a seasonal effect on reproductive performance. One report by Urban and others (19), which involved an analysis of 3,871 litters farrowed between 1944 and 1958, produced no evidence of a station by season interaction although data were included from Madison, Wis., in the north to Stillwater, Okla., in the south. Ahlschwede and Robison (1), in a study designed to determine the influence of climatic conditions (North Carolina) on litter size, reported that they found "little association between climatic conditions at the time of breeding and size of the subsequent litter."

Table 7.--Influence of season on sow productivity (20)

Breeding Date	Number bred	Percent of conception	Average number of pigs	Percent of survival
Apr. 1960	27	67	9.1	88
June 1960	26	81	9.1	88
Sept. 1960	27	56	9.9	89
Nov. 1960	31	90	9.4	97
Feb. 1961	21	95	9.5	82
Apr. 1961	26	92	9.2	80
June 1961	17	71	10.3	82
Aug. 1961	25	64	10.6	90
Oct. 1961	25	84	11.0	90
Dec. 1961	27	96	10.5	86
Feb. 1962	28	89	10.3	91
Total	280	81	9.9	88

Effect of Other Factors on the Pregnant Porcine

Concrete vs. pasture.--Although many observations have been made by swine producers comparing reproductive performance of female swine on pasture and in drylot, few controlled experiments with large numbers of animals have been reported. Wingert and Nelson (23) reported the results from five experiments involving 88 gilt and 92 sow farrowings during two pasture seasons in which the objective was to evaluate the effects of alfalfa-brome pasture as compared to confinement on concrete during flushing, breeding, and gestation. Results obtained (table 8) indicated that treatment differences were small and not statistically significant. Experimental results for conception rate, number of live pigs farrowed, and number of pigs weaned for those confined to concrete as compared to those on pasture were 84 and 87 percent conception; 11.8 and 11.6 live pigs farrowed; and, 9.5 and 9.8 pigs weaned, respectively. Under the conditions of these studies reproductive performance of female swine confined to concrete was equal to those on pasture.

Individual stalls vs. group pens.--Recent interest has been attached to confining pregnant sows to individual stalls rather than to group pens, yet few controlled experiments have been conducted to study the effect of this practice on reproductive performance. European literature likewise fails to provide the desired information. England and Spurr (8) compared the gestation performance of sows and gilts confined to 2 ft. x 8 ft. individual stalls with those in group pens allotted 30 sq. ft. each. Floors were partly slotted and all animals were fed in individual stalls. Average number of pigs born alive per litter was 9.6 and 9.8 for individually confined and group-confined sows, respectively (table 9). Average total number born per litter was 10.5 and 11.1, respectively, for the two treatment groups. Differences between treatments were not significant for the above two traits. Average birthweight per live pig was 2.68 lb. for the individual stalls compared to 2.97 lb. for the group pens--a statistically significant difference. No apparent treatment difference was noted on breeding behavior or conception rate.

A report by Robson (16) indicated that "the greatest single factor against sows tied by the neck or in crates during the gestation period is the failure to observe heat periods." This

Table 8.--Reproductive performance of swine on pasture vs. concrete (23)

Item	Pasture	Concrete
Number of females	90	90
Conception rate, percent	87	84
Average live pigs farrowed, number	11.6	11.8
Average pigs weaned, number	9.8	9.5

Table 9.--Litter size of swine confined during gestation (8)

Item	Sows		Gilts	
	Individual 2 ft. x 8 ft.	Group 30 sq. ft.	Individual 2 ft. x 8 ft.	Group 30 sq. ft.
Average pigs farrowed per litter, number	10.5	11.1	8.6	9.6
Average live pigs, number	9.6	9.8	7.6	8.7
Average birth weight, lb.	2.68	2.97[1]		

[1] Statistically different at ($P<.01$).

limitation was corrected by placing the sows in loose pens or driving a boar behind the individually confined sows daily. Systems of sow management are presently being studied at Purdue University (13) and other Agricultural Experiment Stations designed to determine the most desirable system for maximum reproductive performance.

Sound.--Effects of loud sounds on the physiology and behavior of swine have been reported by Bond and co-workers (4). During three breeding seasons, 10 sows were mated during exposure to jet aircraft sound reproduced at intensities of 120 to 130 decibels. From these trials, it was concluded that swine are almost entirely indifferent to loud sound during mating. Conception rates of sows exposed to sound during mating were equal to those of unexposed sows. Neither the number of pigs farrowed nor the number of live pigs born were influenced by exposure of the dam and boar during mating. To determine whether or not chronic exposure to sound influences later breeding ability, three littermate gilts born in an acoustical chamber and chronically subjected to aircraft sound, were bred and subsequently farrowed with no indication of abnormalities.

Summary

Studies conducted under uncontrolled environmental conditions indicate that approximately 40 percent of all ova ovulated are lost before parturition.

Embryonic and fetal losses largely occur during three periods--the first 25 days, the 60th to 69th days, and the last 21 days.

A 50 percent decrease in viable embryos per 100 corpora lutea was produced when sows were exposed to 98° F. temperatures with 50 percent relative humidity.

Swine exhibit a decrease in heat sensitivity with increasing age of the embryo, and the dam may die of heat prostration before embryonic mortality and abortion occurs.

In limited Studies, 90° F. temperatures failed to reduce fertility of boars.

Under seasonal conditions occurring in Florida, a consistent advantage in conception rate, number of pigs weaned per litter, and weaning weight have been shown when breeding occurred during the cooler months.

Reproductive performance of female swine confined to concrete has been shown to be equal to those on pasture.

Larger pigs at birth from group penned animals was the only significant difference found between gestating animals confined to stalls as compared to pens.

Loud sound in no way affected reproductive performance of swine.

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DISCUSSION

Dr. Whitehair:

It is most appreciated that you have gone over the highlights of this area and not only gave us some of the basic data, but you've used a lot of data from field or practical application. Are there any questions on Dr. Conrad's paper?

Dr. Gustafson:

How many sows were involved in this Purdue Study?

Dr. Conrad:

In this last trial, when we went through the extremes, we had 22, and of this 22, eight died of heat prostration. I think you brought up a good point in that in the Herrick Laboratories, Fred Andrews, physiologist who had been very instrumental in the area of environmental physiology, has conducted studies which I feel are relatively extreme to produce these very significant marked differences.

Dr. Twiehaus:

Would you have some idea as to what the delayed loss might be in the heat studies? In other words, if death may occur following these dates that you gave? I was wondering whether we might not have some aftereffects that would show up later.

Dr. Conrad:

When we slaughter these animals at 25 to 28 days, we cannot say aftereffects. Any comments I might make would be speculation. I would like to defer this one to Dr. Gustafson's presentation, maybe he can elaborate on that.

Unidentified:

What about the effects of the relative humidity?

Dr. Conrad:

I think that we can basically say that any effect that relative humidity has is in direct proportion to the temperature which it exerts. In our particular studies a 50^{O} relative humidity was used. I am sure that if you go up higher in relative humidity you are going to get a more pronounced affect.

Dr. Kernkamp:

On one slide, where you indicated a temperature of 98^{O}, you expressed that 1 day had a very severe effect, but in those which were stressed for 20 days, you didn't have nearly that effect. How do you explain that? Now they all had to have 1 day of stress, but these had 19 more days.

Dr. Conrad:

I'm sorry, I should elaborate on that a little bit. These animals are bred; then they are stressed on day 1 of pregnancy, or day 20 of pregnancy for a period of 24 hours, 48 hours, or 5 days. This is a very significant point.

Dr. Zimmerman:

Was this stress on day 1 immediately following breeding and, when was breeding done in relation to "heat"?

Dr. Conrad:

Dr. Zimmerman, all of these animals were bred twice. When they came in heat in the evening, they were bred in the evening and again in the morning, and immediately put into the environmental chambers. When they came in heat in the morning, they were bred in the morning and again in the evening, then put into the environmental chambers.

Dr. Zimmerman:

Are there figures on the range of implantation time in the sow--the time of implantation?

Dr. Conrad:

The figures that we frequently see is that implantation takes place at about day11. However, this is one of the things that I really made an effort to do, to stay away from the other people's presentations. So here again I think I will let it go at that.

The next speaker is Dr. Twiehaus. Dr. Twiehaus, as many of you know, was Head of the Pathology Department at Kansas State University for a long time, and many of us were exposed to his teaching. In more recent years he has been at the University of Nebraska. While at Kansas, he was always very active on swine diseases research; now he is active in what was underway when he went to Nebraska, including the specific pathogen Free (SPF) program. Dr. Twiehaus will discuss SPF control of uterine infection.

Paper No. 2

S.P.F. CONTROL OF UTERINE INFECTIONS

By M. J. Twiehaus [1]

Most of us here today realize the impact that disease and disease factors and other aspects of gestation have on the economic future of the swine industry. Generally speaking, swine producers cannot do their own research. They must depend upon land-grant colleges and universities, the U.S. Department of Agriculture, and other groups associated with the industry. Our larger swine producers are most apprehensive about the diseases that will be discussed here today -- "The factors producing embryonic and fetal abnormalities, death, and abortion," and they feel like the individuals whose home or business is in the path of a hurricane. They know that sooner or later they will get hit but don't know how soon or how hard.

The term S.P.F.[2] as indicated by Young and co-workers (18) refers to those specific pathogens from which a given population is free. This is accomplished by a break in the swine-to-swine disease transfer. The primary objective is to prevent exposure to and the occurrence of transmissible swine disease. Young (17) presented a method whereby a number of diseases could be eliminated by hysterectomy in one operation. Whitehair and Thompson (14) and Trexler (13) obtained pigs by cesarotomy.

Swine, to be eligible for accreditation under the Nebraska S.P.F. program (see diagram below) must be free from the following diseases: Virus pig pneumonia, atrophic rhinitis, brucellosis, leptospirosis, enteric infections, and external parasites (mange, mites, and lice) (2). Soil-borne disease, such as erysipelas, may not be eliminated but can be controlled by vaccination. On the average farm ascariasis cannot be eliminated but may be controlled by worming procedures.

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[1] Veterinary Science Department, University of Nebraska, Lincoln.

[2] S.P.F. is an abbreviation for specific pathogen-free. The S.P.F. term has replaced "disease-free" in reference to swine repopulation because "disease-free" is absolute, whereas S.P.F. qualifies those diseases that are specifically eliminated or controlled and so defined.

ACCREDITATION REQUIREMENTS--SPF SWINE DISEASE

Disqualify or Conditional	Diagnosis
Virus Pneumonia	Examination at slaughter
Atrophic rhinitis	
Brucellosis	Blood test
Leptospirosis	
Enteric infections	Observation on farm
External parasites (mange, mite, & lice)	

Qualify	Control
Hog cholera	Vaccination
Erysipelas	Vaccination or Antibiotics
Internal parasites	Standard treatment

The S.P.F. program as indicated by Young (17) has greatly contributed to controlling many of the disease problems in our swine, but there are many problems still to be solved (figs. 1, 2).

The key to controlling and eliminating swine diseases through repopulation with S.P.F. stock is in obtaining pigs by hysterectomy from pregnant animals that are immune to certain specific diseases, have not been exposed, and infection is not localized in specific organs (19). Pregnant animals exposed to infection before hysterectomy may be in a stage of septicemia, bactermia, or viremia. Fortunately, most of these pigs die if an infectious or contagious agent is present. Many of the S.P.F. laboratories encountered these problems when pregnant animals were put together and held for several days before hysterectomy.

The incidence and virulence of swine diseases are increased by (1) short generation time, (2) multiple births, (3) number of animals concentrated in a small area, and (4) multiple farrows per year.

These factors all contribute or are related to the problems that we are discussing here today. Data presented by Pinkerton (8), Young (16), Sautter and others (10, 19), and Young (17, 18), have shown the effect or influence of certain viral agents in fetal development. The National Hog Cholera Eradication Program, when completed should materially reduce fetal losses in swine from low virulent field strains of hog cholera viruses and vaccines. Young (17) presented a series of schematic drawings of the developing embryo and indicated that the placenta is not a completed entity until approximately 28 days after conception (fig. 3, A, B, C, D). Data presented by Sautter and others (10, 4), reveal that some modified hog cholera viruses have the ability to invade the embryo during the first trimester and produce abnormalities in immunized animals.

Unpublished work of Moberly[3] at the Nebraska Station indicates that Eperythrozoon suis organism is not capable of infecting the embryo or fetus in the latter stages of gestation in splenectomized gilts. Abortions have been produced in the latter stage of gestation (70th to 80th day when infection was introduced during the first 3 weeks of pregnancy). From present limited

[3]Personal communications.

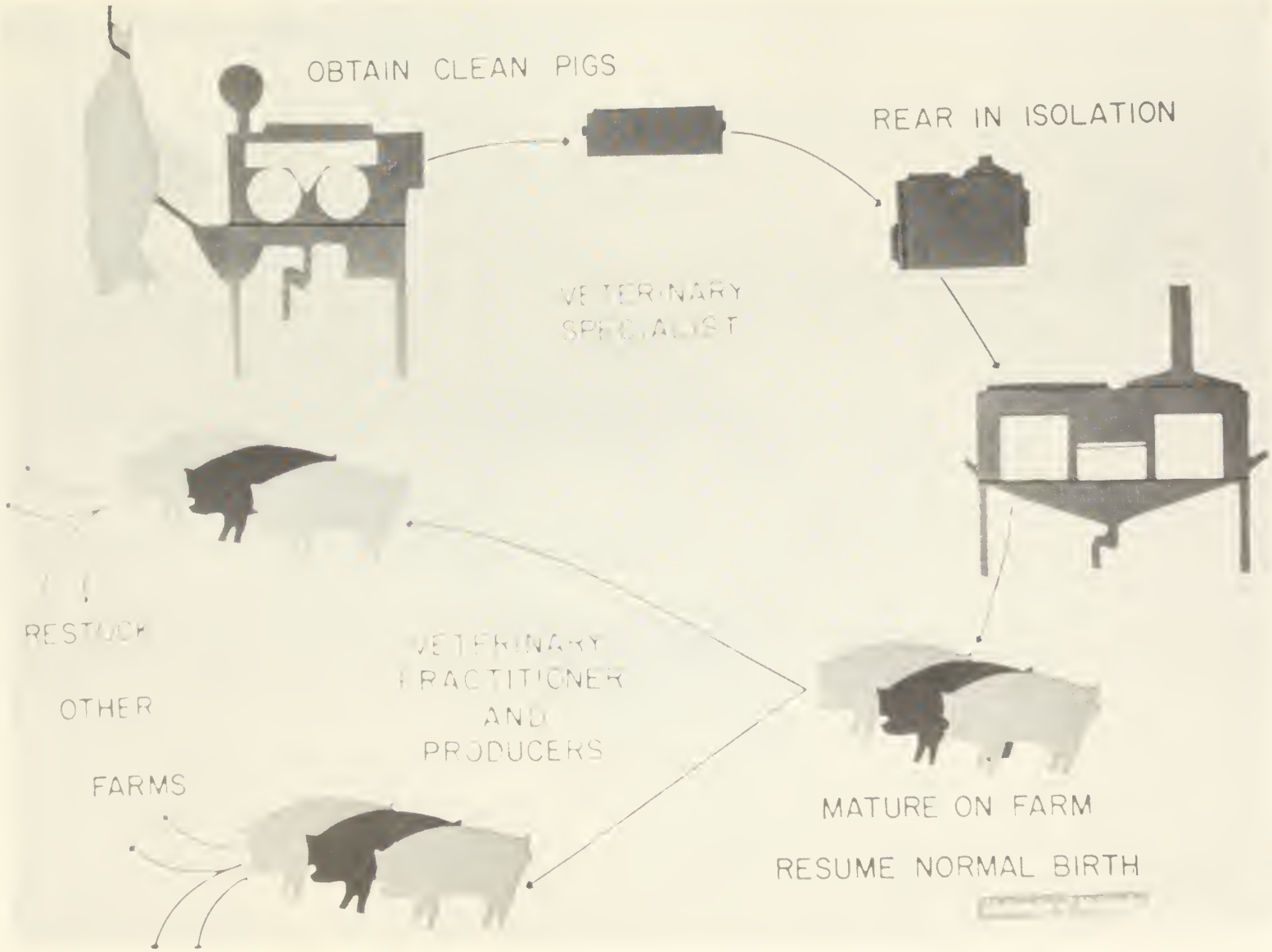
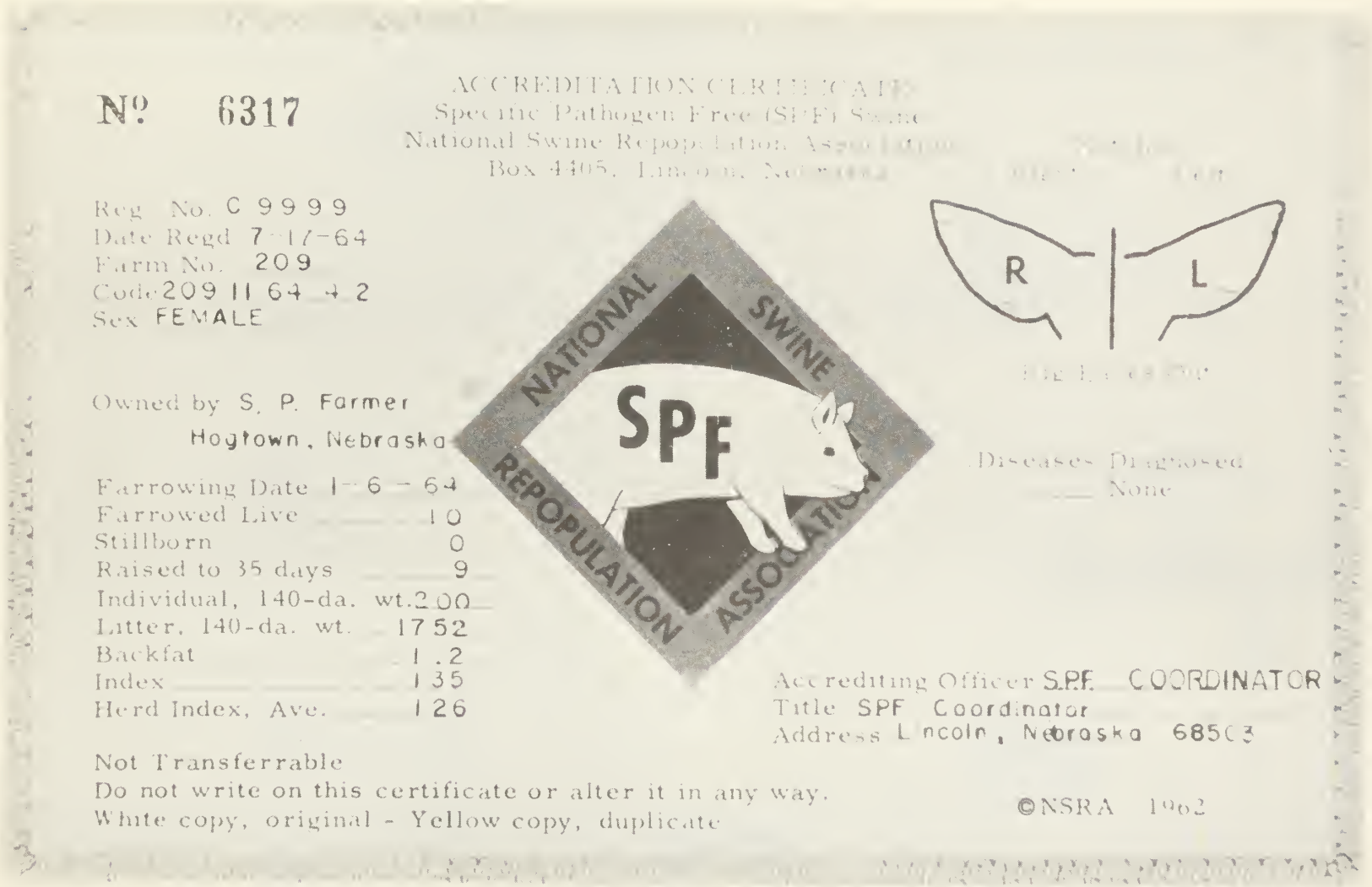


Figure 1.--Scheme for S.P.F.



№ 6317

ACCREDITATION CERTIFICATE
Specific Pathogen Free (SPF) Swine
National Swine Repopulation Association
Box 4405, Lincoln, Nebraska

Reg. No. C 9999
Date Regd 7-17-64
Farm No. 209
Code 209 11 64 4 2
Sex FEMALE

Owned by S. P. Farmer
Hogtown, Nebraska

Farrowing Date	1-6-64
Farrowed Live	10
Stillborn	0
Raised to 35 days	9
Individual, 140-da. wt.	200
Litter, 140-da. wt.	1752
Backfat	1.2
Index	135
Herd Index, Ave.	126

NATIONAL SWINE REPOPULATION ASSOCIATION
SPF

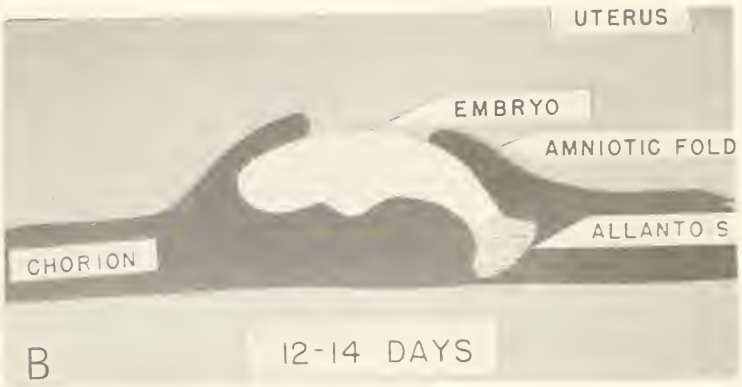
R L

Diseases Diagnosed
None

Accrediting Officer S.P.F. COORDINATOR
Title SPF Coordinator
Address Lincoln, Nebraska 68503

Not Transferrable
Do not write on this certificate or alter it in any way.
White copy, original - Yellow copy, duplicate

Figure 2.--S.P.F. accreditation certificate.



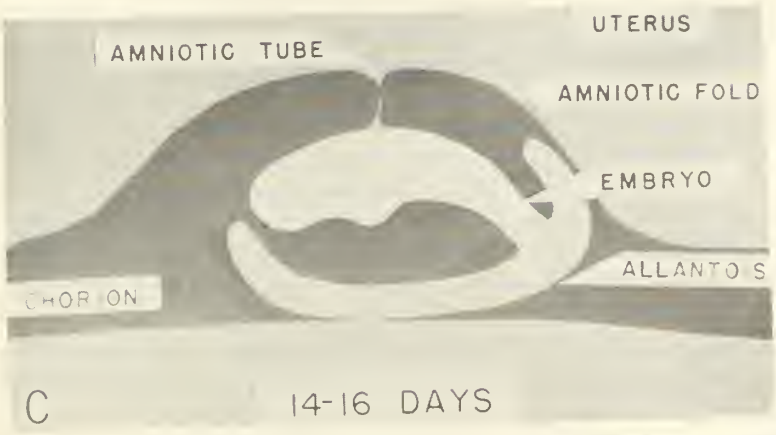


Figure 3.--Schematic drawings of the developing pig embryo at: A, 10 to 12 days; B, 12 to 14 days; C, 14 to 16 days; D, 25 to 30 days.

data it has been concluded that the abortion is caused by anoxia because of the anemia in the sow or toxic factors. Several experimental animals have died at farrowing from parasitemia. Field observations suggest that this infection in late pregnancy may result in sows going off feed at farrowing, become anemic, and result in a reduced flow of milk. The S.P.F. program is thus an effective procedure for eliminating this infection in swine. There is the possibility of the disease being introduced by bloodsucking vectors if infected herds are in the immediate vicinity.

The S.P.F. program incorporates procedures--"Certification of swine herds," and adequate disease control to prevent exposure. Brucellosis has not been a problem and the S.P.F. program is, therefore an ideal means of controlling brucellosis in swine. The S.P.F. program has proven an effective means of maintaining breeding stock free from brucellosis. Blood tests in several S.P.F. herds have revealed nonspecific titers. Upon bacteriological studies and further testing, the herds were again validated.

Transmissible gastroenteritis virus (T.G.E.) to my knowledge does not have the ability to invade the fetus in utero. If it does, no evidence to that effect is left by the virus. Adequate tests are not available for specific detection in such cases. Therefore, as Young and Underdahl (20) reported they were able to bypass this infection in a herd that farrowed continuously by the hysterectomy technique. No cases have been reported in S.P.F. herds during the 8-year period of operation of the S.P.F. program.

Swine dysentery is another disease that may be eliminated and controlled by the hysterectomy technique as reported by Young and Underdahl (20). This has been substantiated by an 8-year study in our S.P.F. herds. One S.P.F. herd, disqualified because of atrophic rhinitis, became infected with swine dysentery. Infection with swine dysentery disqualifies a herd from the S.P.F. program.

Swine influenza is a virus disease that apparently has an affinity for epithelial tissue of the respiratory tract of swine according to Nayak and others (6). Fluorescence was not observed in other organs such as the kidney, liver, and spleen. The abortions and reabsorbed fetuses observed in pregnant animals that sometimes follow an outbreak are difficult to explain as a number of workers (7, 9, 11) have shown that it is unusual for the influenza virus to be transported by the bloodstream. These reports have been supported by the data collected in our S.P.F. herds as no swine influenza cases have been encountered. Thus, this disease may be eliminated by the S.P.F. program.

Leptospirosis is a bacterial disease and is identified by agglutination-lysis tests on serum. Only one herd to date has had positive titers in the Nebraska program over an 8-year period. Vaccine was administered and the titers were negative in the two succeeding pig crops. Carriers apparently did not develop because of the vaccination or by natural immunity. Under the S.P.F. program the incidence of infection with this disease is low and is somewhat difficult to explain when rodents and wildlife are frequent shedders of this organism. No doubt the limited movement of animals into these herds is a factor for the low incidence. The organism has been isolated from aborted swine fetuses by Ryley and Simmons, 1954, in Australia. Further information on this disease will be given by another speaker (see Paper No. 7). The hysterectomy technique will eliminate this bacterial disease as young pigs readily overcome infection during isolation period before the pigs are placed in breeders.

Pseudorablies (Aujeszky's disease) has not been observed in swine under the S.P.F. program. Young (15), Gordan and Luke of Ireland (3), Terpstra (12), and Akkermans of Netherlands (1) reported pseudorabies as a cause of embryonic deaths in pigs. A field observation in 1965 by Twiehaus[4] in a commercial swine herd experiencing embryonal deaths in some litters was confirmed by the fluorescent antibody test to be pseudorabies virus in aborted feti. This observation was confirmed by Stair[5] that pseudorabies virus is capable of causing fetal death and reabsorbed feti when pregnant animals were inoculated intranasally after 70 days' gestation. Earlier intranasal exposure failed to elicit the clinical picture described above. Sows inoculated

[4] Unpublished data.
[5] Personal communication.

became clinically ill but recovered in all cases but one. Some pigs were not infected in a litter when the sows were experimentally inoculated in the latter stages of pregnancy. With these observations and a field case in a commercial herd, surgical procurement of pigs will apparently not bypass this infection and is not a means of controlling or eliminating this virus infection in swine.

Metritis-mastitis syndrome in pregnant sows and gilts is not well understood at the present time. Mycoplasma has been isolated by Moore and others (5) 1966, as the causative agent from this syndrome. They were able to reproduce clinical cases with the isolate. Abortions in one herd in 1965 was suspected with mycoplasma infection. Isolations were not made and treatment with broad spectrum antibiotics proved of value. Subsequent farrowings have not revealed a similar clinical picture. Evidence to date is not available as to the possibility that this disease agent could be eliminated and controlled by the S.P.F. program.

Summary

The specific pathogen-free (S.P.F.) program has been widely accepted by many swine producers primarily because of the elimination of specific diseases.

The S.P.F. program has carried with it better management and disease control. The system emphasizes disease control by preventing the transfer of diseases from one animal or herd to another. Additions to herds in the program must originate from other S.P.F. herds.

The S.P.F. program has been an effective procedure for eliminating transmissible gastroenteritis, influenza, swine pox, swine dysentery, eperythrozoon suis, brucellosis, leptospirosis, and other certain bacterial agents. Experimental data at the Nebraska Station and field evidence indicate that hog cholera virus and pseudorabies virus cannot be eliminated by this program.

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DISCUSSION

Dr. Whitehair:

Dr. Twiehaus, you have certainly given us the highlights of the S.P.F. program. I do hear both sides of the specific pathogen-free program, some criticism at times; but I think the whole program has done a lot to focus emphasis on disease aspects of swine. The S.P.F. program has also brought out at times the importance of animal to animal spread. In so many instances of disease spread we tend to blame blackbirds and everything else, and we ignore our animal to animal spread. You certainly emphasize that. Another speaker, at a meeting some years ago in Michigan, mentioned that this is a technique to monitor the type of infections we have in some of our herds. I think this is certainly a valuable tool. Do you have any questions to ask Dr. Twiehaus?

Dr. Dunne:

Dr. Twiehaus, would you mention what the effects of roundworms are in the S.P.F. herds, and do you have a problem in this regard?

Dr. Twiehaus:

Yes, Dr. Dunne, we do have problems with roundworms in the S.P.F. herds. It is not possible to keep these S.P.F. herds clean as far as roundworm infestation is concerned. When the program was originally set up, animals were placed on farms which we knew had not had swine on them for a good many years. Despite this, about the end of the first year we found evidence of ascarid infestation in the swine. We put a lot of emphasis on parasitism in our herds primarily because we feel it does play an important role as far as disease aspect is concerned. We ask our fieldman to make checks on our S.P.F. herds three times during every farrowing season to collect fecal samples from these animals on the farm and bring the specimens back for examination. So, we keep our fingers on the ascarid population in our S.P.F. herds very well. We believe they should worm their animals, but once in a while they become complacent and think the ascarids are not causing any particular problem. Certainly the work that Dr. Underdahl has done on the influence of the ascarid larvae in influenza would warrant attention directed to the ascarid population.

Dr. Dunne:

Would you care to comment on the possibility of intrauterine transmission?

Dr. Twiehaus:

In the work we have done, we have not been able to demonstrate that this occurred in swine, despite the fact that it has been reported in other animals. We have found no evidence that intrauterine infection might occur from ascarid larvae.

Unidentified:

In what location in the fetus did you find immunofluorescence specific against pseudorabies virus?

Dr. Twiehaus:

Particularly in the liver, brain, and spleen; the spleen was probably the most ideal tissue in the feti for this observation.

Dr. Dunne:

Was your incidences of swine influenza about the same in the S.P.F. herds as in your regular swine production?

Dr. Twiehaus:

No. We are looking for it to happen, but we have not. I think here again that the animal to animal transmission is quite important.

Dr. Shuman:

Have you had any experience with abscess in any of these S.P.F. herds?

Dr. Twiehaus:

The answer to that is yes. This has been a problem of great concern to us in our S.P.F. program. In fact, we have suspended some of the herds because of this. Two herds in particular, several years ago, had a lot of problems with jowl abscesses. I assume this is what you have reference to. Further work needs to be done in this particular area. By treatment, we have been able to bring this under control, but it takes a long time. We kept one herd suspended for over a year; we didn't want him selling any breeding stock.

Dr. Dunne:

For the benefit of those of us here, we should call on the gentleman who suggested a name for the disease to tell us what he thinks it should be called.

Dr. Twiehaus:

I would welcome a suggestion. Dr. Shuman, what would you want to call this infection?

Dr. Shuman:

Call it abscesses of swine, then if you find the cause tack that on.

Unidentified:

In the 8-year study of the S.P.F. program, have you noted any marked improvement in the reproductive performance of the S.P.F. over the regular?

Dr. Twiehaus:

I can say no to that. This has remained fairly constant.

Dr. Whitehair:

We have a few minutes left. Are there any further questions you would like to ask Dr. Twiehaus? I hesitate to put a colleague and friend on the spot, but at times it seems to me we use different terminology--, for example, S.P.F. and germ free. Dr. Waxler would you care to comment on terminology as far as germ free? This is an entirely different technique namely, what we call the "closed system," in which the pigs are not exposed to the outside. If you have any comments on the terminology, some of us would be interested. This is probably a technique more for research workers than the applied. We've lived with this technique primarily in laboratory animal areas, but I do think that some of you in research might be interested in it.

Dr. Waxler:

I don't have any particular comment other than what you have said. The germ-free technique is certainly one that has a different purpose in mind. In other words, we are raising animals for a specific disease research where we expose these animals to only one organism--in a sense, a closed system, or in a pure-culture concept perhaps as opposed to the S.P.F. pigs which is for herd repopulation. So our purpose is certainly different along this line.

Dr. Whitehair:

And then we use the term gnotobiotic for one organism or two or three or four.

Dr. Dunne:

Does Dr. Waxler or anybody have any comments on the possibility of virus-free, germ-free pigs?

Dr. Waxler:

This is something you don't get people to comment on even when they are raising gnotobiotic or germ-free laboratory animals, as far as the freedom from virus infection. This is something on which there is apparently not much data. Evidently there are some viruses that are being transmitted in utero. This is some of the work that has been done at Notre Dame in laboratory animals, rats, and mice, possibly they are transmitted in utero. Possibly not. So from the standpoint of swine I wouldn't care to make any comment on it. Because as far as I know there is no data one way or another on it. Dr. Whitehair, you mentioned the term gnotobiotic. This is a new term used to indicate an animal that is completely free of demonstrable bacteria. These animals are then exposed to one certain type of organism, and we use this technique to study the effect of exposure. These animals are exposed to one particular organism such as Escherichia coli, but it is still a gnotobiotic animal. We know what the exposure is, but it is not a germ-free animal. So, it is a differential terminology to designate this difference.

Paper No. 3

NUTRITION AS IT AFFECTS INTRAUTERINE LOSSES

By Dwane R. Zimmerman[1]

[PAPER NOT AVAILABLE FOR PUBLISHING]

[1]Animal Science Department, University of Nebraska, Lincoln.

ENDOCRINE AND EMBRYOLOGICAL FACTORS AS RELATED TO EARLY EMBRYONIC LOSS IN SWINE

By P. J. Dziuk[1]

Early embryonic loss in swine is characterized by unseen resorptions of embryos rather than a visible abortion and usually by loss of part of the litter rather than the whole litter. In most studies the proportion of eggs fertilized has been reported to be near 95 percent. The number of embryos at 30 days of gestation is about 60 to 70 percent of the number of corpora lutea. Assuming each corpus luteum represents one ovulation point and hence a potential embryo, this 30- to 40- percent loss has a major influence on litter size at birth (4, 9, 10, 15). The following review will be primarily of recent research reports related to embryonic losses in swine and literature cited in these reports.

Effect of Hormones

Endocrine deficiencies have been proposed as one of the possible causes for losses of early embryos. The pregnant sow undergoes considerable adjustment in hormone levels and ratios to accommodate the embryos. Any slight maladjustment could cause loss of the embryo that is so dependent on the proper maternal hormonal status.

Several experiments have been conducted in an attempt to determine the effect of supplemental estrogens or progestogens on embryonic loss (5, 6, 7, 14, 18, 20, 21, 22, 24). In a few instances there was some hint that exogenous steroids may have reduced embryonic loss but subsequent experiments have not borne this out. To date there is no concrete evidence to support the idea that embryonic loss is caused by endocrine deficiencies, or that supplemental hormones will lessen the embryonic loss. Continued research may produce knowledge of the levels and ratios of hormones necessary for optimum survival of embryos so that hormones could be given so they need not ever be limiting if indeed they are.

Effects of Gametes

Eggs and sperm have finite lives that are not lost suddenly but are lost by gradual senescence (20). Sperm stored for 24 to 96 hours has been shown to be capable of fertilizing an egg and initiating development. Such eggs, however, are less likely to develop into viable embryos than eggs fertilized by fresh sperm (8, 20). Eggs aged several hours after ovulation before fertilization by delayed mating or insemination have a higher incidence of polyspermy and other forms of abnormal fertilization than eggs fertilized immediately after ovulation (12, 13). While these aged eggs are fertilized, they are less likely to develop into fetuses. Aged gametes appear to be possible contributors to embryonic loss.

The sow remains in heat for about 40 to 48 hours during which time she may be mated or inseminated. Ovulation occurs near the end of heat. The time of insemination relative to stage of heat, and consequently relative to time of ovulation, has been shown to have a very pronounced effect on conception rate and litter size (3, 23). Insemination either early or late in the heat

[1]Animal Science Department, University of Illinois, Urbana.

period gives a lower conception rate and smaller litter size than insemination in the middle of the heat period. Because it is not readily apparent, how insemination time could affect the number of eggs ovulated, the reduced litter size suggests either fewer eggs were fertilized or a greater embryonic loss occurred or perhaps both.

The boar may have a greater effect on embryonic loss than is commonly thought (2). In comparison of conception rate and litter size among several different boars, boars with a conception rate of less than 65 percent to one service had a greater proportion of very small litters than those with conception rates of 65 percent or more (3). There were marked differences among boars in the conception rates and average size of litters they sired. The boar, therefore, is also implicated in embryonic loss.

Number and Position of Embryos In Utero

Pregnancy in swine will rarely continue to term if the number of embryos is very small (16), or if half or less of the uterus is occupied by embryos.[2]

After reducing the number of embryos in gilts during the first or second day after estrus, gilts may be divided roughly into three classes based on the response to treatment. When no fertilized eggs remained in the uterus, the interval to next estrus was 21 days. When one to four presumably fertilized eggs were left in the uterus, the next estrus occurred in 25 to 30 days. When four or more embryos were left, pregnancy continued to term (16). One to four fertilized eggs can extend the interval between heats but cannot maintain pregnancy.

Restriction of embryos to certain segments of the uterus at the first 10 days of gestation resulted in failure of pregnancy when one-half or more of the uterus was unoccupied. When one-third or less was unoccupied, pregnancy continued. Killing embryos in one-half of the uterus before day 10 resulted in failure of pregnancy while pregnancy continued when fetuses were killed at day 12 or later.[3] These data suggest that the pregnant pig in some way "counts" her embryos and determines their position in the uterus when making the necessary physiological adjustments to pregnancy. When the number of embryos is small or when they are not distributed throughout the uterus, the pregnancy is stopped, embryos are resorbed, and heat recurs. This tends to reduce the incidence of very small litters except those arising from late fetal death.

Uterine Space per Embryo

Intrauterine crowding of embryos has been suggested as a possible cause of embryonic loss in the pig (1, 17) and other species (9). A series of experiments changing the proportion of the uterus available to each embryo has indicated that within the limits of the usual number of embryos the space available per embryo had no effect on embryonic survival rate.[4] Reducing the number of embryos to half the usual number by ligation of one oviduct did not increase the proportion surviving. Restriction of migration of embryos by ligating the uterus and causing inequalities in the space available to each embryo did not affect embryonic survival. Removing one uterine horn with its adjacent ovary reduced uterine space available but did not affect embryonic survival.

The number of embryos in a litter has been increased by superovulation (11) and by transferring additional eggs[5] suggesting the uterus can hold more than the usual number of embryos. Other workers (1) have not been able to show an increase in embryo numbers from transfer of more eggs. While there is some evidence showing that intrauterine crowding is not the limiting factor in embryo survival, additional research will be required to finally resolve the question.

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[3] See footnote 2.

[4] Dzuik, P. J. Effect of number of embryos and uterine space on embryo survival in the pigs. Unpublished. 1967.

[5] See footnote 4.

Conclusion

Evidence of the cause(s) of the usual 30 to 40 percent embryonic loss before day 30 of gestation in swine is not sufficiently conclusive to encourage one to make final statements. At this time the loss seems innate and not reduced by any one treatment or management scheme. The losses seem to be caused primarily by errors associated with fertilization resulting from union of gametes capable of fertilization but perhaps deficient in one or more of the thousands of genes needed to produce a differentiated embryo and fetus. Superimposed on this background of innate loss may be other factors that may contribute to embryonic loss. These losses are known to exist but the exact nature of their cause is still largely unknown.

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DISCUSSION

Dr. Ulberg:

I would like to hear your comments on how many of the pigs are carried to term?

Dr. Dziuk:

I don't know, but I would guess about 14. We have superovulated a number of gilts and have been able to get an average litter size of nearly 15. These are not old gilts either. We usually deal with them in either their first or second heat. I think they probably have room in there if other things are right. What the other things are I don't know. If we knew, we could do something about them. I think Ron Hunter showed that he could get 18 embryos on an average in sows. So I feel there is more room in there so to speak. How much room I don't know, surely there is a limit someplace. I would guess it could vary between gilts and depend on other factors. What the other factors are I don't know.

Dr. LeJeune:

Does repeated natural servicing during the same issuing period increase the embryonic mortality and does repeated natural servicing increase the rate of fertilization?

Dr. Dziuk:

In answer to the first question, I would guess it would not, because you would be more likely to inseminate or mate the animal during the optimum time when she should be mated. This is likely to increase the embryonic survival. The same thing is true of your second question as far as fertilization or conception rate. If you mate several times you are more likely to hit the optimum time than if you do it just once. You are more likely to increase both conception rate and embryonic survival rates.

Unidentified:

On this optimum servicing time, what are we talking about in terms of hours?

Dr. Dziuk:

We are talking about a period of not more than 10 to 12 hours at the most. We have done some work where we double-mated animals at a peak time relative to ovulating. When we inseminate a Duroc gilt with a Duroc boar, we get red pigs; use a white or Yorkshire boar, and get white pigs. So, at 6-hour intervals we inseminate first with a Duroc and then with a Yorkshire, and at some other time during ovulation use another combination. The data we have obtained this way support the data we see here. That is, if you inseminate later than about 20 to 24 hours before ovulation, the first boar settles nearly every pig. You use the Duroc first or the Yorkshire first, it really doesn't make much difference. But, if insemination is earlier than 20 to 24 hours before ovulation, you get a mixture between the two animals.

Paper No. 5

BREEDING AND GENETICS AS RELATED TO INTRAUTERINE DISTURBANCES IN SWINE

By John F. Lasley[1]

Losses of potential young between ovulation and parturition, particularly in swine, have been of great interest to research workers. These losses have been of interest because of their high incidence and because of their great economic importance.

Intrauterine death losses are due to a complexity of causes. Some are caused by environmental factors, such as a disease that may act directly upon the developing young; whereas, others may be caused by indirect effects upon the young through the maternal environment. Some intrauterine losses are either genetic in nature or involve genetic material. These include chromosome aberrations, lethal genes that affect the fetus directly, and certain incompatibilities between the uterine environment supplied by the mother and the developing young. Some of these genetic maternal affects may not become evident or have their final effect until after birth.

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The Frequency of Intrauterine Losses in Swine

Total losses between ovulation and parturition in swine are easily determined. This can be done by simply slaughtering the pregnant female at the desired time and counting the number of corpora lutea on the ovary (represents the number of ova produced) and the number of fetuses in the uterine horns. The difference represents total losses of potential fetuses during this period, and they are usually termed embryonic death losses.

Data summarized in table 1 show that these losses represent about 30 to 35 percent of the total ova ovulated. For the technical standpoint, these losses include more than embryonic death losses that occur before the embryo has developed and implanted. Oxenreider and Day (23) were able to recover only 418 ova out of 492 ovulated (corpora lutea count on the ovary) in the fallopian tubes of 46 sows and gilts slaughtered within 2 to 4 days after breeding. This represents only an 85 percent recovery rate. It compares to an 80 percent recovery rate reported by Squiers and others. (28). Ova not recovered could include those not released from the follicle, those lost in the abdominal cavity and those which could not be flushed from the oviduct and uterine horns. Some of those not recovered undoubtedly would include a certain proportion of normal, fertilized ova; but we can only speculate as to what proportion this would be.

The fertilization rate of ova in swine where fertilization of at least one egg occurs appears to be close to 95 percent (Squiers and others, 28). This is based on the percent of ova recovered from the reproductive tract which showed cleavage. Possibly those not recovered had a lowered fertilization rate.

The difference between the number of corpora lutea found on the ovary and the number of embryos present in the reproductive tract should not be referred to as embryonic death losses. If we deduct the 5 percent of the ova not fertilized and assume that about one-half of the ova not recovered never developed into fertilized eggs, true embryonic death losses would be more nearly 15 percent of the total eggs ovulated than the 30 to 35 percent often reported.

The failure of fertilization and the failure of fertilized eggs to reach the implantation stage could be due to both environmental and genetic factors. Again, we can only estimate the proportion of each of these factors involved. The proportion would undoubtedly vary from one mating to another.

Table 1.--Prenatal death losses in swine in different experiments

Workers	Stage of gestation observed	Percent of corpora lutea not represented by embryos at slaughter
Hammond (13)	14 to 60 days	32.6
Corner (8)	Different stages	30.0
Squiers and others (28)	25 days	35.0
Lerner and others (18)	17 days	25.1
Do	25 days	33.6
Rathnasabapathy and others (25)	55 days	31.3
Reddy (26)	55 days	30.1

Prenatal Wastage Due to Chromosome Aberrations

The development of techniques for studying chromosome numbers and structures in cells from tissue cultures has shown a number of chromosome abnormalities in humans. More recently these techniques, or modifications of them, have been used in studies with swine. Studies at the Missouri Agricultural Experiment Station with both swine and cattle have failed to show any chromosome abnormalities in certain normal and abnormal individuals. Workers at other stations, however, have reported some chromosome aberrations in isolated instances.

Aneuploidy.--This is a type of chromosome abnormality in which the individual possesses either 2n + 1 or 2n - 1 number of chromosomes. It also includes abnormalities of more than

2n + 1. In 1963, it was observed that a Swedish Landrace boar when mated to 21 sows produced average litters of 5.6 pigs as compared to 12.7 pigs from the same sows bred to other boars (Henricson and Backstrom, 15). This represents a reduction in litter size of about 56 percent. A culture of blood leucocytes from this boar showed that about three-fourths of one chromosome (from either pair four, or pair three) had been translocated to one of the chromosomes of pair 14. As a result, approximately one-half of the progeny of this boar received only one-fourth of the chromosome (four or three). Apparently the missing genes usually carried on the missing part of the chromosome were vital to the developing embryos, causing their death either when the egg was fertilized or at least early in its development. Individuals such as this boar are probably extremely rare, but these results do show that chromosome abnormalities can be related to prebirth losses in swine.

McFeely (20) studied chromosome numbers in blastocysts from the mating of two unrelated Yorkshire boars to seven gilts of various breeds. A chromosome analysis of the parents from peripheral blood leucocytes showed no abnormalities. In addition, no physical defects were noted in the parents. When a chromosome analysis was made from the blastocysts among the progeny, 10 percent of those collected possessed grossly detectable chromosome defects and 2.3 percent were already degenerating as shown by the absence of mitotic figures and the great preponderance of pycnotic cells. Many of the chromosome karotypes showed triploid and tetraploid numbers of the X and Y chromosomes. These results suggest that this type of chromosome abnormality may be responsible for about one-third of the prenatal wastage of ova as determined by differences between corpora lutea number on the ovary and the number of blastocysts recovered.

Polyploidy.--Polyploidy refers to cells, or individuals, that possess exact duplicates of the haploid chromosome number other than the diploid number. This would include individuals that had the 3n, or more, number of chromosomes in body cells. In mammals, polyploidy almost always results in the death of the individual, although the developing fetus may survive to mid-pregnancy.

The condition of polyploidy where the individual has three or more complete sets of chromosomes may be brought about in different ways (Austin, 2). A fertilized egg may have two or more male pronuclei in the cytoplasm in addition to the usual one female pronucleus. Proof of this in swine is based on the finding of two or more sperm tails within the cytoplasm. This condition is known as polyspermy and has been observed in swine. Hancock (14) observed that the rate of occurrence of polyspermy is related to the age of the ovum when it is fertilized. None of the 34 ova recovered from sows mated during the first part of estrus showed polyspermy. When sows were not mated until 30 hours after the beginning of estrus, seven out of 56 ova (12.5 percent) showed polyspermy. Where matings were delayed until 48 hours after the beginning of estrus, 12 out of 29 (41.4 percent) were polyspermic. Thibault (29) also reported an increase in the incidence of polyspermic ova when matings were delayed.

These results suggest that when aged ova are fertilized, polyspermy is more likely to occur. Evidently, there is a breakdown in aged ova of the formation of the fertilization block which normally prevents the entrance of more than one sperm into the cytoplasm.

Polyploidy may occur in still another way in swine. An ovum may possess two female pronuclei and one male. An ovum normally possesses only one female pronuclei, but apparently under certain conditions there can be a breakdown in the process of meiosis, allowing two instead of one nucleus of occupy the cytoplasm of the ovum. Thibault (29) reported triploidy (two female and one male pronuclei) in 21 percent of the eggs recovered from sows mated 36 hours or more after estrus began.

Since the estrous period in sows normally lasts 2 to 3 days and ovulation can occur over a period of several hours in some individuals, a proportion of the eggs from such matings could be polyspermic because the aging of the ovum before fertilization took place. This could account for some prenatal death losses.

Baker and Coggins (3) reported a high rate of polyspermic ova in prepuberal gilts (160 to 180 days of age) injected with various levels of pregnant mare serum gonadotropin (PMS) followed by the injection of 500 I.U. of human chorionic gonadotropin (HCG) 48 hours later. Thirty

immature ova collected from these gilts had an average of 145 sperm in the zona pellucida, 42 in the vitelline space, and 11 in the ooplasm. Their results suggest that polyspermy also occurs when immature ova are released from the ovary and are exposed to spermatozoa (fertilization). Apparently, immature ova have not yet acquired the ability to block polyspermy.

Transferrin Types and Reproductive Performance in Swine

Evidence of possible incompatabilities between the fetus and maternal environment has been found in swine. Kristjansson (16) studied the breeding records of 530 Landrace and Yorkshire gilts mated to 44 Yorkshire and Landrace boars. Yorkshire gilts mated to Landrace boars gave the highest return rate; whereas, there was no significant difference between the other three possible matings between these two breeds. The same data were divided into the nine mating classes (transferrin genes A & B) based on transferrin types of the boars and gilts. Differences in the proportion of returns to service between these nine mating types were significant ($P<.01$). When (BB) boars were mated to (AB) sows the return rate was 46 percent as compared to an overall nonreturn rate of 25.3 percent ($P<.01$). Differences in return to service for the eight other transferrin matings were not significant. These results suggested that there was an incompatability between the (BB) boars and the (AB) sows. A closer study of the data supported the theory that the high return rate of this type of transferrin mating was due to a higher early embryonic mortality rather than a failure of fertilization. Apparently, a fetus-maternal environment incompatability was involved.

Prenatal Death Losses Inherent in the Fetus

We will consider here the effect of specific genes in causing intra-uterine death losses in swine. Evidence for such effects may be found in reported cases of lethal genes in the literature and in litter size at birth as affected by the degree of inbreeding or crossbreeding of the fetus.

Lethal Genes.--Lethal genes are those that cause the death of the individual up to and including the time of birth. The effects of such genes probably occur any time between fertilization and birth. Specific examples of lethal genes reported in the literature include those in which an obvious defect in anatomy occurs at birth in a certain proportion of the pigs in a litter. Many other lethals may cause prenatal deaths, but their presence is not observed nor suspected unless a post-mortem examination is preformed.

Some reported lethal genes and their proposed mode of inheritance are summarized in table 2. Most lethal genes are recessives because if they were dominant, they would kill the individual carrying the gene and he would leave no offspring through which the gene could be transmitted.

No detrimental or recessive sex linked genes have been reported in swine. Many sex-linked detrimental recessive genes have been reported in humans. Recent chromosome studies show that the X chromosome in swine is much larger than the Y and thus carries more genes as is

Table 2.--Some reported lethal genes in swine

Name of defect	Probably mode of inheritance	Reference
Atresia ani	Duplicate dominace	Berge (4)
Hydrocephalus	Recessive	Warwick and others (32)
Paralysis of hind legs	do	Berge (4)
Cleft palate	Unknown	McPhee and others (21)
Thickened forelimbs	Recessive	Walther and others (31)
Muscle contracture	do	Hallqvist (12)
Fetal mortality	do	Eaton (10)

true with humans. There is no reason to believe that sex-linked (detrimental) genes do not occur in swine. They have not been reported perhaps because no one has taken the time to study inheritance of such traits in swine. Determining the cause of deaths in human infants is usually studied in detail, especially if there is a possibility the cause is inherited. Deaths in baby pigs are usually accepted as the usual thing without any bother to determine their cause. Careful research should reveal several sex-linked recessive traits in swine.

Inbreeding and Line Crossing Effects in Pigs Upon Their Intrauterine Survival.--The main genetic effect of inbreeding is to increase the homozygosity of genes. What this means is that for any two genes in a population (such as genes A and a) inbreeding increases the proportion of individuals that are homozygous dominant (AA) or homozygous recessive (aa) or both.

When the two recessive genes pair up in an individual, they express themselves phenotypically. Since most recessive genes are detrimental, one would expect some detrimental effects to appear. This is exactly what happens when inbreeding is practiced in swine. The paired detrimental genes vary in their effect from one that kills the individual early in embryonic life to one that may not kill the individual but may decrease its vigor, especially shortly after birth.

A study of pooled data from several experiment stations (Dickerson and others, 9) showed that for each 10 percent increase in inbreeding of the pigs there was a corresponding decrease of 0.20 pigs per litter at birth. This effect occurred even when the dam was not inbred and shows that recessive, or partly dominant, genes in the homozygous state were causing intrauterine death losses in some pigs. Similar results have been reported by Squiers and coworkers (28), Godbey and Godley (11), Bradford and others (5), and Urban and others (30).

Linecrossing and crossbreeding effects also indicate whether or not genetics play a part in intrauterine death losses in swine. Linecrossing and crossbreeding have the opposite genetic effect of inbreeding in that these systems of mating make more pairs of genes heterozygous within a particular population. Thus, theoretically, they should reduce embryonic death losses because greater heterozygosity should result in more vigor. Several experiments show an increase in litter size at birth when pigs from a two-line cross are compared for litter size with pigs from the two pure lines, or when litter size in the two-breed cross is compared to litter size in the two pure breeds that make up this cross. This limits the effects of linecrossing and crossbreeding to those inherent in the pigs and not to the sows that produce them. (See Bradford and coworkers, 5; Chambers and Whatley, 7; and Lasley 17).

The heritability of litter size at birth in swine varies between 0 and 20 percent (Urban and others, 30, and Noland and others, 22). This suggests that genes with an additive effect have very little effect on intrauterine survival. The detrimental effects of inbreeding and the desirable effects of line crossing and crossbreeding on litter size at birth strongly suggest that genetic effects on intrauterine survival are nonadditive in their expression.

Pisano and Kerr (24) made a study of lethal equivalents in a herd of Poland China swine. They defined a lethal equivalent as a value corresponding to a single lethal gene with a 100 percent probability of causing the death of an individual, or to two genes with an average 50 percent probability of causing death, or to three genes with a one-third probability of causing death, and so forth. They calculated that the Poland China swine with which they were working had 1.63 lethal equivalents per animal affecting embryonic life.

Inbreeding and Linecrossing Effects in Sows Upon the Intrauterine Survival of Their Pigs.--Something within the maternal environment supplied by sows can affect the intrauterine survival of their pigs. We don't know the exact nature of this effect, but we know that it can be influenced by heredity. In the study by Dickerson and others (9), the effects on litter size at birth caused by inbreeding of the pigs and that caused by inbreeding of the dams were separated. When this was done, there was still 0.17 fewer pigs at birth for each 10 percent increase in the breeding of the sow. Similar results were observed by Urban and coworkers (30).

Chambers and Whatley (7) noticed an improvement in litter size at birth in linecross sows as compared to pureline sows, and Lasley (17) summarized results showing that there was a considerable advantage in litter size at birth for crossbred over purebred sows. Some of this advantage, however, was due to a higher ovulation rate in linecross and crossbred sows. Squiers

and others (28) by slaughtering gilts at the 25th day of pregnancy, found that much of the advantage in litter size of linecross over pureline sows was due to a higher ovulation rate ($P<.01$), although there were also fewer intrauterine death losses in linecross sows ($P<.05$).

Day[2] conducted an interesting experiment which shows that the uterine and post-uterine environment (a part of which is genetic) supplied by the sow can have an influence on the survival and vigor of her pigs. An inbred line of Yorkshire swine was developed at the Missouri Agricultural Experiment Station for the purpose of studying the physiological basis of inbreeding. As inbreeding increased to 40 to 50 percent in this line, fertility became so poor that it was difficult to produce enough pigs to maintain the line. In winter of 1966, two inbred Yorkshire sows were bred to an inbred boar from the same line. Twenty fertilized ova were obtained from these two sows and were transferred to two purebred Hampshire gilts. Fifteen pigs were born and survived until about 10 days after farrowing when five of them died. Ten uniform and thrifty inbred pigs have survived to the present time. Since inbreeding effects are genetic effects, these results suggest that the genetic environment supplied by inbred sows may not be optimum for the survival of inbred pigs. Further research is needed, however, before definite conclusions can be drawn as to the possible causes.

Post-natal Losses Related to the Intrauterine Environment

Between 30 and 35 percent of all pigs farrowed die before weaning (Smith, 27; Godbey and Godley, 11). Most of these losses occur within the first 4 days after farrowing. Between 6 and 7 percent of the pigs born are dead at birth (Lynch, 19; Brekke, 6). Some of these losses are caused by intrauterine disturbances before birth resulting in the birth of weak or dead pigs. Some of these deaths are due to genetics and some are due to environment, or to a combination of both.

The best evidence that some of such losses are genetic in nature is found in inbreeding and crossbreeding results. Increased inbreeding is accompanied by an increased death loss of pigs between birth and weaning (Bradford, 5; Urban, 30; Dickerson, 9). These increased losses of pigs occur with the increased inbreeding of the pigs as well as the sows. There is evidence that something more than a poorer ovulation rate makes the inbred sow a poorer mother than a noninbred or crossbred sow. Some of this difference (but not all) can be ascribed to the poorer milk-producing ability of the inbred sow.

Crossbred sows, in general, wean larger litters of pigs than purebred or inbred sows even when the pigs from both groups of sows are crossbreds. A part of the larger litter size in crossbred sows is an advantage in ovulation rate and in less embryonic death losses. There appears to be an advantage for the crossbred mothers, however, over and above that because of a higher ovulation rate and greater milk production. What this advantage might be is difficult to determine, but some of it could be from a prebirth environment. At least a part of this must be genetic. These losses are a little different from the occurrence of hemolytic disease in pigs that occurs only after the pigs consume the causative antibodies in the colostrum, or first milk (Andresn and Baker, 1).

Summary and Conclusions

Total wastage of ova in swine before birth ranges between 30 and 35 percent. These losses are not all caused by embryonic death, however. When gilts and sows are slaughtered and ova are flushed from the reproductive tract, usually only 80 to 85 percent of the ova, based on corpora lutea counts, are recovered. Some of the ova not recovered probably never leave the follicle and some are lost in the abdominal cavity or other parts of the reproductive tract. About 5 percent

[2] Day, B. N. Transplantation of ova in swine. Personal communication. 1967.

of the ova are not fertilized, or at least do not show cleavage. Probably only 15 to 20 percent of the ova losses can be truly classed as embryonic death losses. Some of these losses could be genetic in nature.

Chromosome abnormalities can cause intrauterine death losses. One case has been reported in which a Landrace boar heterozygous for a translocation of a chromosome produced litters about one-half normal size. A study of blastocysts of the pig indicate about 10 percent had chromosome abnormalities. Polyploidy may also result from the fertilization of premature or aging ova by more than one spermatozoa (polyspremy).

Incompatability between the mother and fetus may be responsible for some prenatal death losses as shown by transferrin studies. Other prenatal death losses may be from the presence of lethal genes that cause the death of the developing fetus before birth or afterward. Inbreeding increases prenatal death losses; whereas, crossbreeding decreases them. This evidence, together with low heritability estimates for litter size at birth, suggests that genes with a non-additive phenotypic expression affect prenatal death losses. This evidence does not indicate how many genes may affect intrauterine losses nor does it suggest their specific causes or effects.

Considerable evidence suggests that many losses between birth and weaning may be caused by intrauterine disturbances before birth, some of which may be genetic in nature.

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Unidentified:

Would you clarify the terms, "fetal death" and "embryonic death"? In the literature this is sometimes confusing.

Dr. Lasley:

I have been taught, maybe it was too long ago, but there is a period of ovum, there is a period of embryo, and there is a period of fetus. The period of fetus, of course, is when the organs and the various body parts have already been formed. I think we could agree on this, maybe it would be a good thing to discuss.

Dr. Dunne:

I would like to discuss this particular aspect. We have the same problem of trying to determine when it was an embryo and when it was a fetus. We have used the development of the skeleton as being the stage when it ceases to be embryo and becomes a fetus. At this point it can no longer be absorded if it dies, and at this point we have considered it a fetus. I would like the reaction of the group as to when it ceases to be an embryo.

Dr. Lasley:

It is like the question, "When does a pullet become a hen?" But I do think this would be as good a method as any. I think that all we have to do is agree on where the point is and I certainly go along with it.

Dr. Abrams:

I would like to ask Dr. Lasley if he ever thought of the possibility that feed additives may be effective in prenatal death losses.

Dr. Lasley:

I am glad you asked me that Dr. Abrams. I'd like to talk to you privately about it. I don't think estrogens affect reproduction at all, to any extent. Why, because I think it is a balance, a balancing kind of gene action rather than an additive type. Why? because you have to have a balance between FSH (follicle-stimulating hormone) and LH (luteinizing hormone) to get ovulation. Another thing, how long have they selected for larger litter sizes in swine? I guess since we have had swine. Now Dr. Abrams disagrees with me, but we can do this privately and come to an understanding between the two of us.

SECOND SESSION: H. W. Dunne,[1] Chairman

Paper No. 6

ACQUISITION OF IMMUNOLOGIC COMPETENCE BY THE PIG[2]

By W. L. Meyers[3]

The pig at birth is essentially devoid of any serum-globulin and normally acquires large amounts of maternal-globulin from the colostrum. If newborn pigs are deprived of colostrum, their level of serum-globulin does not reach the level of colostrum-fed pigs until about the 7th week of life (11). Colostrum-deprived pigs have been found to be relatively immunologically incompetent when compared to colostrum-fed pigs of the same age (2, 9, 10, 11). These special characteristics of the newborn pig make it uniquely suited to the study of the acquisition of immunological competence in the absence of maternal-globulin and to assessment of the effects of various globulins on antibody formation. Evidence will be offered that the immunological competence of pigs is dependent on the presence of preformed antibody. This antibody is initially of maternal origin but is later produced by de novo synthesis in the individual. The evidence presented will be interpreted on the basis of the natural selection hypothesis of antibody formation. According to this hypothesis, which was proposed by Jerne (3, 4) and later elaborated by Eisin and Karush (1), the proper antigenic stimulation is induced by the antigen-antibody complex and not the antigen alone. The immunologically competent animal would, therefore, have in its circulation natural antibodies, formed in the absence of antigenic stimulation, that are capable of combining with those antigens to which the animal can respond.

The degree of antibody response by colostrum-deprived baby pigs seems to be somewhat dependent on the nature of the antigen used. Sterzl and others (13) found no differences in the antibody response of colostrum-free and colostrum-fed baby pigs when several particulate antigens such as sheep erythrocytes, bacteriophage, and Salmonella paratyphi B. were used. Kim and others (6) reported an excellent antibody response to MSP-2 actinophage by germfree, colostrum-deprived, miniature baby pigs. In contrast, Staub and Boguth (11) reported that colostrum-deprived pigs were unable to respond measurably to Brucella abortus before they were 10 weeks old. Hoerlein (2) found that colostrum-deprived pigs failed to respond immunologically to B. abortus, bovine serum albumin, and chicken egg conalbumin and responded only poorly to sheep erythrocytes after antigenic stimulation at 3 weeks of age. Staub and Boguth and Hoerlein reported a greater antibody response in colostrum-fed than in colostrum-deprived pigs.

A lack of antibody response to diphtheria toxoid given to day-old or 3 week-old colostrum-deprived pigs was reported by Segre and Kaeberle (9, 10). They also reported that feeding of colostrum enhanced the antibody response by baby pigs of similar ages.

Another factor that seems to effect the immunologic ability of newborn colostrum-deprived pigs is previous immunization of the sow with the same antigen used later in her pigs. Heorlein (2)

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[1]Department of Veterinary Science, Pennsylvania State University, University Park.

[2]Some of the investigations described in this paper were supported by contract Nonr-1834 (37) with the Office of Naval Research and by contract COO-1628-2 with the Atomic Energy Commission.

[3]College of Veterinary Medicine, University of Illinois, Urbana.

found that colostrum-deprived pigs from sows previously immunized with sheep erythrocytes were able to give an immune response to that antigen, whereas, colostrum-deprived pigs from nonimmune sows were not. A similar result was reported by Myers and Segre (8), who found that newborn, colostrum-deprived pigs whose dams had been actively immunized with diphtheria toxoid were capable of an antibody response to the toxoid, while pigs from nonimmune sows were not. Furthermore, antibodies against diphtheria toxoid were found in sera of the newborn colostrum-deprived pigs delivered by hysterotomy. This finding was offered as evidence for transplacental transfer of antibody and as a possible explanation for the specifically increased immunologic competence of colostrum-deprived pigs from immunized sows. Although Šterzl and others (12) and Kim and coworkers (6) reported finding no trace of antibodies in newborn, colostrum-deprived pigs from either actively or passively immunized sows there remains the possibility of transfer of antibody in quantities that could not be detected by the tests employed. To increase the chance for detection of very small quantities of globulin an experimental method has been devised by Myers and Segre using allotype specificity rather than antibody function as a marker for the γ-globulin. In this way the total amount of γ-globulin transferred can be measured rather than that small proportion of γ-globulin molecules that have a particular antibody specificity. Experiments using this method are now underway and should help to elucidate the question of transplacental transfer of antibody globulin in swine.

The enhancing effect of this very small amount of transplacentally transferred maternal γ-globulin on the immunologic ability of the newborn pig may be interpreted on the basis of the natural selection hypothesis of antibody formation as previously described (1, 3, 4). Thus, a small amount of maternal γ-globulin acquired transplacentally by the colostrum-deprived baby pig would supply sufficient natural antibody to form complexes with the injected antigen and initiate antibody formation.

Even though the quantity of transplacentally transferred maternal γ-globulin is very small in swine, it nonetheless will be in direct proportion to the concentration of the antibodies of various specificities in the maternal circulation. Therefore, a proportional rise in a single antibody specificity in the maternal circulation will be reflected in a higher rate of transplacental transfer of that specific antibody to the fetuses. The newborn, colostrum-deprived pigs with an increased amount of maternal γ-globulin of this specificity would be more capable of responding to the injected antigen of the same specificity according to the natural selection hypothesis.

The enhancing effect of preformed antibody on antibody production by colostrum-deprived baby pigs was demonstrated by the work of Segre and Kaeberle (9, 10). They found that colostrum-deprived pigs failed to respond immunologically to diphtheria toxoid given at birth or during the third week of life. However, if the diphtheria toxoid was mixed with dilute specific antiserum before injection, the animals were able to produce appreciable amounts of antibody. It is important to note that Segre and Kaeberle used extremely small quantities of specific antibody to obtain the enhancing effect. The quantity was so small that no specific antibody was detectable by passive hemagglutination with the dilution of antiserum used. It is well known that passive immunization to an antigen often inhibits active immunization to the same antigen as long as sufficient quantities of passive antibody remain in the circulation. However, the amount of antibody inhibiting antibody formation would appear to be much greater in most cases than that quantity used by Segre and Kaeberle (9, 10) for enhancing antibody formation.

The reports of Šterzl and others (13) and Kim and others (6) of failure to detect any trace of antibody in colostrum-deprived pigs that later gave a very good antibody response does not disprove the role of pre-existing antibody in antibody formation. The level of pre-existing antibody necessary to initiate antibody production can be quite small and well below the level of detection by the usual immunologic tests. Šterzl and others (13) and Kim and others (6) did not report what effect small quantities of passive antibody, such as Segre and Kaeberle (9, 10) used, had on antibody production for the antigens they employed. These same workers did not relate what effect pre-immunization of the sow with a given antigen had when the same antigen was

given to the colostrum-deprived baby pigs from that sow. The inclusion of this type of experiment would have permitted a direct comparison of the effect of passive antibody on the antibody response to the particulate antigens used by Sterzland coworkers (13) and Kim and coworkers (6).

The nature of the preformed antibody which controls the immunologic competence of newborn pigs has been further elucidated by the work of Kaeberle and Segre (9, 10) and Locke and others (7). These authors reported that 7S, γ2 gloublin (IgG) antibodies were capable of enhancing antibody formation, but 19S, γ1M (IgM) antibodies were not. This finding was significant in light of the model of antibody production of Eisen and Karush (1). According to this model, only bimolecular complexes (Ag Ab) would be immunogenic, while multi-molecular complexes (Ag_2 Ab or Ag Ab_{n+1}) would not be immunogenic. The system, therefore, depends upon a bivalent antibody molecule, a well-recognized property of IgG antibody. Significant new support of the Eisen and Karush model is found in the recent work of Kerman and others (5). They reported that newborn mice from mothers immunologically tolerant to pneumococcal polysaccharide were significantly more susceptible to the induction of immunologic tolerance and immunity with the polysaccharide than newborn mice from normal mothers. These results are exactly those that could have been predicted on the basis of the Eisen and Karush model. The offspring of immunologically tolerant mothers would not receive any passive maternal antibody corresponding to the tolerated antigen. The newborn mice would have in their circulation only their own natural antibodies corresponding to the tolerated antigen and would, therefore, require less of the specific antigen to induce immunity or immunologic tolerance than newborn mice from normal mothers.

The newborn pig's relative immunologic incompetence seems to be a consequence of its deficiency in natural antibody. The quantity of natural antibody needed for immunologic competence appears to be small. The level of competence can be significantly increased by small amounts of passive antibody. This deficiency in natural antibody is normally corrected by passive acquisition of maternal antibody from the colostrum. The level of natural antibody will also increase in the pig by spontaneous de novo synthesis. In this way the pig will in a few weeks acquire complete immunologic competence.

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DISCUSSION

Unidentified:

Has anyone ever tried to use antigen on the sow and boar before breeding to see if that will change the amount of natural antibodies?

Dr. Myer:

You see, by definition, you just destroyed the concept of natural antibody. Remember my early definition was--natural antibody had to be produced in the absence of any stimulation. We can modify the immunologic competence of the animal, which is normally acquired for its production of natural antibodies, by simply giving it antibody some other way. This can be done by immunization of the mother. The mother then transmits this antibody to the fetus. The fetus then is no longer dependent upon that small amount which it can produce by de novo synthesis, the knowledge it acquired by an extra amount from the mother; therefore, this pig's competence is going to be increased over a pig from a nonimmunized mother.

Dr. LaSalle:

I would like to know something about the procedure used to obtain the 10 X concentration. Also, you once said, possibly by a slip of the tongue, that paralysis and tolerance are the same. I think there is an important difference between tolerance and paralysis. First, tolerance is what occurs when you inject an antigen in the animal in its fetal life, before it is born or in its early neonatal life. At that point, the animal recognizes this antigen as its own protein or body antigen and fails to recognize it as a foreign protein--a foreign agent. It takes a large quantity of the same antigen in this animal and it will not produce any reaction to it. That is the opposite of what you have in a paralyzed animal that can respond to a very small quantity of antigen. I believe they are about diametrically opposed in that respect. Do you agree with this?

Dr. Myer:

Well, no, not exactly. I'll answer the first question, then try to answer your second one. The concentration was obtained very rapidly, with the cold ethanol procedure, which we were using at that time. We achieved the globulin. The globulin was simply lyophilized and then the total amount of a given volume of serum was then reconstituted to give essentially one-tenth of the original volume of what we started with. That was the 10X concentration procedure. The difference between immunological paralysis and tolerance I've heard debated before. In my mind they aren't different. I will have to explain that the part in which I may have gotten you slightly confused is this thing on mice.

Tolerance or paralysis simply means a nonresponding animal. The mechanism by which the animal does not respond is exactly the same in both cases. If it is a case of the animal not responding to self, it is simply because self has been present in a concentration which is always equal to antigen excess. According to the theory of Eisen and Karush, antigen excess does not produce immunity. Now they carry it even further to say, of course, that during neonatal development that actually an excess of certain proteins apparently causes a suppression of any further development of those clones of cells which would produce the so-called natural antibody. So we can attack it from either angle. I was not intending to show that the mice from tolerant mothers are not tolerant--that wasn't the idea at all. The tolerant mothers are immunologic paralyzed mothers and are in fact complete nonresponders. In other words, these mothers don't respond to this antigen at all. They are immunologically paralyzed. They have no serologic response at all. The only thing I was trying to show here is that we are not getting active paralysis in these little mice. We went through a lot of laborious procedures to show that there was no antigen present in these mice when they were born or even in fetuses. So this is not the mechanism that is working here. The only mechanism that is at work, we postulate, is simply that the mice from paralyzed mothers don't receive any antibody from the mothers, but that is specificity, since the mothers have none in circulation because they are immunologically paralyzed so they have none of the antibodies in circulation. Therefore, they give none of that to their offspring. So, the offspring is entirely dependent upon that which it has been able to make of its own, natural antibody.

Dr. LaSalle:

In what respect is that different from the mice from the nonimmunized mothers? They don't receive any antibody for that particular substance either.

Dr. Myer:

Nonimmunized mothers?

Dr. LaSalle:

Yes, the mice from normal mothers. They're not immunized. They would not receive any antibody.

Dr. Myer:

Oh, yes they do, because the mother has in circulation her normal complement of natural antibody produced in the absence of antigenic stimulation.

Dr. LaSalle:

But they are nonspecific?

Dr. Myer:

Oh no; they are all quite specific. There is probably, if you postulate, about ten thousand specificities available in this pool. So if you are going to believe the Eisen and Karush logic, you have to believe that the animal has in circulation some small population of antibody molecules which are specific for that given antigen you are working with.

Dr. LaSalle:

You mean that the specific number of antibodies that could take care of that particular antigen would eliminate it?

Dr. Myer:

In paralyzed mothers, it would be eliminated. Therefore, they have none to give to their offspring. The normal mother has a little bit to give which is apparently enough to make the difference. That's the whole idea.

Unidentified:

What about thymectomy?

Dr. Myer:

The big work in this area has been done in mice by Miller where he showed that if you thymectomized mice, essentially within 24 hours of birth, the mice failed to develop immunologically with a complete look of competence as compared with unthymectomized litter mates. The only thing this is dependent upon is the species of animal and how functional the thymus is before birth. In certain strains of mice apparently, the thymus can get enough done before birth that the animal essentially doesn't miss the thymus after birth. In other strains it doesn't. In other words, apparently the development of immunologic competence goes on in different breeds and different species at a different rate. The pig apparently is immunologically competent enough as far as the thymus is concerned that thymectomy of the pig doesn't make that much difference. Whatever function the thymus has, it is essentially carried out before birth.

Dr. LaSalle:

If a mouse born from an immunologically paralyzed mother receives absolutely no antibodies that are fit for this particular antigen, how come that a very small minute dose of this antigen would immunize that mouse?

Dr. Myer:

Well, it's just a matter of quantities. Now if we believe this, that we need the biomolecular complex that is the correct immunological stimulus, then it's the complex of antigen and antibody.

Dr. LaSalle:

Right, this is what I don't understand.

Dr. Meyer:

Okay, let's put it on this basis then. Now let's assume that the mouse from the normal mother has in circulation a 100 molecules of this antibody that it needs. Therefore, if we give it the correct amount of antigen so that we have a 100 molecules of antigen, then we have the 100 biomolecular complexes that are the correct stimulus. But the mouse from the immunologically paralyzed mother doesn't have 100 molecules, maybe it has 10. So, if we attempt to give it 100 molecules of antigen, which is the normal immunizing dose, you see what happens--we don't get biomolecular complexes, we get complexes in the range of antigen excess. So we get all these Ag2-Ab complexes that are not the correct immunologic stimulus. Therefore, we have to give the animal 10-fold less antigen than is the normal immunizing dose for the mouse. So instead of giving it 100 molecules we give it 10. Now these match up with the 10 molecules that it has. We have the biomolecular complex and the correct stimulation.

Dr. LaSalle:

In other words, the paralyzed mothers did pass on some of those?

Dr. Myer:

No, No! This within the mouse, is de novo synthesis in the mouse itself, in the baby mouse. The baby mouse is making some of its own. In the normal mouse you have a combination of what it has made of its own and what it got from its mother. So it is an ad lib effect.

Paper No. 7

LEPTOSPIROSIS

By O. H. V. Stalheim[1]

The first report of the isolation of leptospires in swine was from Australia in 1939 (22). In 1946, Gsell (19) reported the leptospiral etiology of "swine herd's disease," a well-recognized syndrome among persons who raised swine in Switzerland. In 1949, porcine leptospirosis was described in Argentina (31). Gochenour and his associates in 1952 (18) first described leptospirosis in the United States, when Leptospira pomona was recovered from an explosive outbreak in association with hog cholera and Salmonella cholerasuis. Since that time, our concept of swine

[1]Animal Disease and Parasite Research Division, Agricultural Research Service, USDA, National Animal Disease Laboratory, Ames, Iowa.

leptospirosis has evolved from that of a highly fatal infection with icterus, encephalitis, and death to that of a widespread, usually inapparent infection that is caused by several antigenically distinct leptospires, and manifested almost entirely by reproductive disturbances (4). Except when enhanced by other factors, the economic cost of swine leptospirosis is represented by embryonic and fetal death, neonatal death, and infertility. But, in addition, leptospiral infections are zoonoses.

Swine are considered to be a primary host for the most prevalent pathogenic leptospire in animals, L. pomona. By urinary dissemination, they are a frequent source of severe illness in man with uveitis (3) and paralysis (12) as sequelae. Another leptospire, L. grippotyphosa, was isolated recently from a cow following an abortion (20). This leptospire, which is very virulent for man, (3, 6) is well established in cattle, swine, and wild animals in Illinois.[2] After a brief description of the pathogenesis of leptospirosis, I will discuss some factors influencing the severity of the disease on swine embryos and fetuses and some methods for controlling losses from swine leptospires.

Based on observations of naturally occurring field cases and on experimental infections (7, 11, 16, 18, 25, 27, 30, 32, pp. 143-151, 33), the course of leptospirosis in pregnant sows may be described in figure 1. In experimental infections, leptospiremia may be detected as long as 10 days after exposure with slight and transient fever usually occurring about day 5 (33). In natural cases, however, the clinical signs may be severe, and high fevers, prostration, convulsions, icterus, and high mortality has been described (18). With the appearance of antibodies in the blood (about day 5), leptospires begin to disappear from the blood and all organs except the brain, eye, and kidney. They persist indefinitely in the kidney (24) and in the brain for 18 days (34), but the persistence of leptospires in the aqueous humour of swine has not been adequately investigated. With regard to the effects on pregnancy, leptospirosis in swine may be inapparent or disastrous depending upon several factors.

One of the most important factors determining the outcome of a leptospiral infection in pregnant sows is the stage of pregnancy at the time of exposure. This fact is illustrated by some results of a recent experiment at the National Animal Disease Laboratory. In connection with the evaluation of an experimental vaccine for protection against leptospiral abortion, six sows in different stages of pregnancy were exposed to renaltropic strain of L. pomona by injecting 5 ml. of culture (about 2×10^9 leptospires) intramuscularly into each sow on 2 successive days. Twenty-six days later, they were killed and examined for evidence of renal and fetal leptospirosis (table 1) (39).[3] The kidneys of all sows harbored leptospires and contained gross lesions (11) typical of renal leptospirosis. The fetuses of sows in early pregnancy (estimated 60 days) apparently were normal. Two fetuses were cultured for leptospires with negative results. Fetal death and resorption occurred in two sows in advanced pregnancy; the fetuses were not cultured. One sow, that was pregnant 90 days at the time of exposure, aborted nine dead pigs 11 days post-exposure. The pigs were estimated to be 1 to 2 weeks premature. Six to 14 hours after delivery, the livers of three of the pigs were cultured for leptospires with negative results.

These results may be explained as follows. As pregnancy advances, the mounting nutritional demands of the rapidly growing fetuses are met by an increased production of fetal chorionic villi and by a thinning of the membranes interposed between the fetal and maternal circulations(5). Since this change results in increased permeability of the membranes and permits a greater exchange of nutrients, it can be assumed that it also facilitates the penetration of leptospires into the fetal circulation. Consequently, after about 56 days of pregnancy, even a brief period of leptospiremia may serve to infect all the fetuses of a gravid uterus. The sheer mass of dead and devitalized fetuses will assure their prompt expulsion. If most of the sows are in a similar stage of pregnancy, a "storm" of abortions will result.

If the sows are in a less advanced stage of pregnancy, massive invasion of the uterus may kill all the fetuses, but rather than being expelled they will be resorbed (table 1). Although tremendous

[2] Personal communications.

[3] Stalheim, O. H. V. Vaccination against leptospirosis, safety, potency, duration of immunity and protection against leptospiral aboryion with viable, avirulent Leptospira pomona. Amer. Jour. Vet. Res. (Submitted for publication).

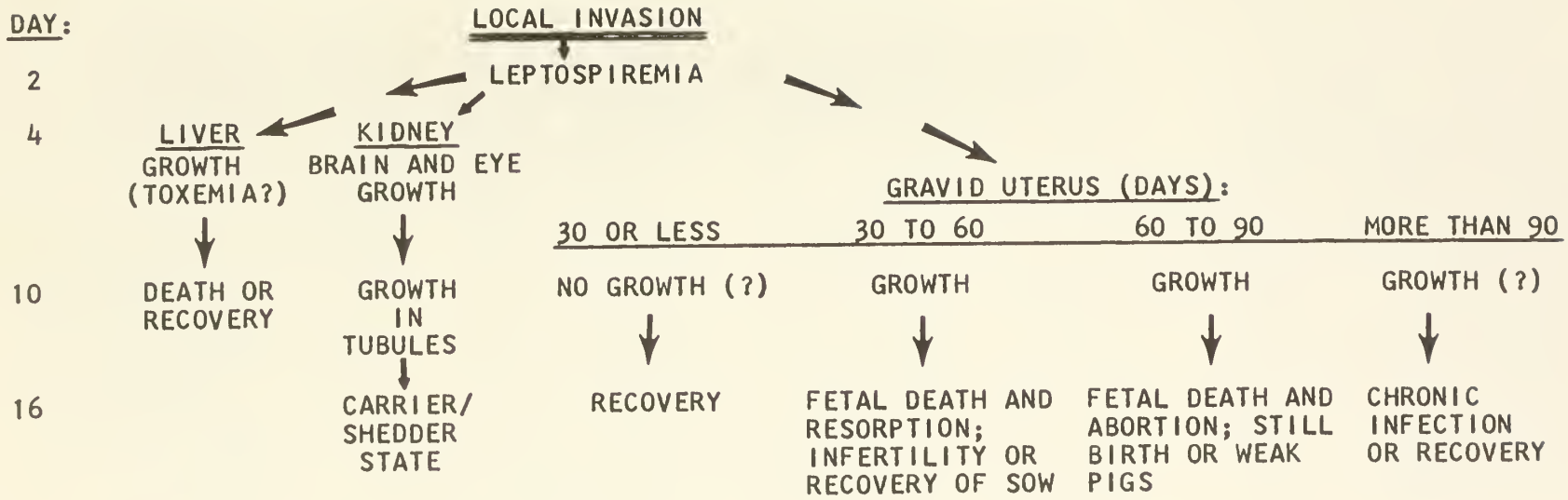

Figure 1.--The course of leptospirosis in swine.

Table 1.--Experimental leptospirosis in pregnant swine

Gilt No.	Days of pregnancy	Gross lesions	Renal localization	Effect on feti
1	60	Moderate	Yes	None.
2	60	Severe	do	None.
3	60	Very severe	do	None.
4	60-90	Severe	do	Death and resorption.
5	60-90	do	do	Do.
6	90	do	do	Death and expulsion.

Sows in different stages of pregnancy were exposed with Leptospira pomona and observed for 26 days; then they were killed and examined for evidence of renal and fetal leptospirosis.

numbers of leptospires (up to 2 x 109 per grain of tissue) are present in the kidney or liver of infected swine (39), the cellular response is surprisingly slight. If the effects of one or more dead fetuses do not suffice to stimulate the uterus to contraction, with dilation of the cervix, and expulsion of the contents (that is, abortion), the dead fetuses will be partly or completely resorbed.

We do not know the relative frequency of fetal death with resorption compared to fetal death with abortion, but presumably, abortion is the most extreme and less frequent manifestation of leptospirosis in pregnant sows. Leptospiral abortion did not result in permanent sterility in sows (26), but the cost of temporary infertility because of resorption must be considerable. Recent studies in Holland (1, 2) revealed repeat breeding, infertility, and abortion in about half of the swine breeding heards. The main cause was infection with L. hyos (L. tassari).

The failure of L. pomona to kill swine embryos (table 1) is in agreement with the experiences of other investigators. However, the reports of the Dutch workers suggest that we should re-examine this aspect.

The second factor determining the outcome of leptospirosis in pregnant sows is the virulence of the leptospires. Fennestad and Borg-Petersen (15) exposed four groups of pregnant sows (74 ± 3 days) to L. pomona., L. sejroe, L. icterohaemorrhagiae, and L. saxkoebing by intravenous injections. The latter two serotypes did not initiate renal infections and had no detectable effect on the fetuses. L. sejroe caused leptospiruria, and when the piglets were born, almost half of them were weak or nonviable. However, L. pomona caused renal and fetal leptospirosis with abortion approximately 3 weeks after inoculation, or 2 to 3 weeks before term. Because some strains of L. pomona are more invasive for the kidney than other (renaltropic) (35), it seems reasonable to assume that some strains of L. pomona are more invasive for swine fetuses than others. But such differences have not yet been demonstrated.

The basis for differences in virulence among strains of leptospires is not known. Leptospiral virulence does not appear to be related to toxicity of the leptospires or to their lipolytic or hemolytic activities, but virulent L. pomona has phospholipase activity whereas avirulent L. pomona does not (38).

The outcome of leptospirosis in pregnant sows also depends upon interactions of the classic factors of the host-parasite relationship. Depending upon the stage of pregnancy, the virulence of the leptospires, the degree and duration of the exposure, and the resistance of the sow and the fetuses, most of the latter may escape infection and develop normally. However, leptospires penetrate the placental barriers of other fetuses and gain access to the fetal circulation. In a few days, tremendous numbers may be observed in the blood and organs of infected fetuses (28). In swine, leptospires neither localize in the placenta nor cause extensive placentitis (17). After death, infected fetuses may be partly or completely resorbed.

In addition to transplacental infection, swine fetuses may be infected with leptospires from an adjacent fetus. Thus the infection may be transmitted from one fetus to another within the uterus (15). Intrauterine transmission of leptospirosis is apparently a rather slow process and may continue until the intervention of parturition. Consequently, the litter may contain apparently healthy pigs as well as examples of all stages of fetal disease. Although neonatally infected pigs may recover and grow normally, some authors have described stunting and unthriftiness (18). Neonatally infected pigs have shed leptospires for a least 42 days (17). Studies on their immunological responses to leptospiral antigens have not been reported.

Whether a swine fetus has "resistance" and may recover from a leptospiral infection is not know. Bovine fetuses produced specific antibodies after exposure to leptospires (14). A comprable phenomenon has been observed with swine fetuses,[4] but a comparable phenomenon has not been reported with swine fetuses. The reticulo-endothelial cells of adult guinea pigs actively phagocytize leptospires, even without the assistance of agglutinating antibodies (13), but the phagocytic capability of the fetal reticuloendothelial system has not been investigated.

The losses from leptospirosis in pregnant sows are summarized in table 2. Embryonic or fetal death is not a constant sequel (15, 17). An infection during the first month of pregnancy is usually without gross effects on swine embryos. Infection during the second month most often causes fetal death and resorption, while infection during the third month of pregnancy usually causes abortion within 10 to 38 days. Infection during the last 3 weeks of pregnancy causes little or no ill-effects to the fetuses. Upon ingestion of specific antibodies in the colostrum, the baby pigs may recover completely.

Thus in pregnant sows, the outcome of leptospirosis depends upon several factors, of which only a few have been recognized and even fewer have been appraised. In both the sow and her fetuses, the protean manifestations of leptospirosis range from inapparent infection with complete recovery to acute illness and death. In other species of animals and in man, the clinical signs are still more diverse (3), and the determination of causal relationships has been delayed.

In addition, the possibility exists that some fetal deaths and abortions of unknown etiology are actually caused by leptospires as yet unrecognized in this country. Antibodies to these "foreign" leptospires would not be detected with our present antigens, and attempts to isolate them in laboratory media may fail for several reasons. First, the lipid contained in body tissues is inhibitory to the growth of leptospires (36). Consequently, leptospiral growth may be completely inhibited in the presence of low dilutions of lipid-rich renal or portal tissue (1:100 or less) (17). Secondly, some fastidious leptospires grow poorly or not at all in artificial media (20). Finally, contaminating bacteria frequently "overgrow" any leptospires that may be present in certain diagnostic specimens, such as aborted fetuses. This difficulty can be largely overcome by adding 5-fluorouracil to the growth medium to inhibit the growth of contaminating bacteria (23).

In discussing the prevention of perinatal losses from leptospirosis, I shall briefly discuss artificial immunity and chemotherapy and omit sanitation, quarantine, and so forth. Chemically

Table 2.--Immunity to renal leptospirosis in swine[1]

Immunogen	Number infected	Number challenged	Immunogen	Number infected	Number challenged
A cured infection	0	5	-Irradiated vaccine	2	14
Commercial bacterin	16	16	Streptomycin vaccine	0	7
Experimental bacterin	14	17	Viable avirulent vaccine	5	74

[1] After swine were vaccinated 2 to 3 weeks with 5 ml. of the immunizing agent, their immunity was challenged by two injections (5 ml.) of renaltropic L. pomona. Two weeks later, they were killed and cultures taken for evidence of renal leptospirosis. (Data taken from references 40, 41).

[4] Fennestad, K. L. Royal Veterinary and Agricultural College, Copenhagen, Denmark: Personal communication, 1967.

killed cultures of leptospires will protect swine against the clinical forms of leptospirosis including fetal death and abortion; but evidence is accumulating (40) that they do not confer immunity to infection and initiation of the renal-carrier-condition (renal leptospirosis). The data summarized in table 2 indicate that immunity to renal leptospirosis was conferred by leptospires that were rendered nonreplicating by exposure to ionizing radiation or streptomycin (40) or by vaccination with living avirulent strains (41).[5] Strains of avirulent leptospires may be selected by growing virulent leptospires in a synthetic (protein-free) medium (37). So far, the avirulent strains have not regained their virulence when administered to hamsters, guinea pigs, cattle, or swine. If leptospirosis appears in nonvaccinated, pregnant sows, the losses can be minimized by vaccinating and by administering antibiotics to the sows. The intramuscular administration of dihydrostreptomycin (25 mg./kg. in 1 dose) will destroy any leptospires in the sow or the fetuses (21) including those leptospires localized in the kidney (39). Chlortetracycline in the feed (400 g./ton of feed for a week) should also stop losses that are caused by leptospirosis, although at this level, L. pomona will not be eradicated from the kidneys (39).

The prevention of fetal losses from leptospirosis might be facilitated in certain situations by chemoprophylaxis. For example, swine may shed L. pomona without detectable seroagglutinins (6, 11). Thus, it might be well to consider the prophylactic administration of antibiotics to breeding animals before they are introduced into leptospirosis-free herds or into the United States from foreign countries.

Chemoprophylaxis might also be a useful adjunct to our present serologic procedures. After an infection, leptospiral agglutinins may persist in swine for at least 2 years (24) and in cattle for 7 years (29). Rather than lose the services of valuable foundation animals that have residual serologic titers, they could be treated prophylactically (39)[6] and returned to service.

Chemoprophylaxis and more effective immunizing agents should be useful tools for preventing the entrance of new leptospires into this country, for controlling the dissemination of leptospires among swine, to other animals (including man), and to minimize losses in pregnant swine.

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DISCUSSION

Dr. Kernkamp:

Today, we have heard a few comments on clarification for the terms "embryonic deaths" and "fetal deaths." I would like to clarify another, and that is the term "stillbirth." I think all of us have an idea of what a stillbirth is--a lifeless born fetus would be a stillbirth. Oftentimes, we have a need to record certain births as stillbirths. Instead of using this term I would like to sug-guest we use the term "perinatal deaths." Literally such a term would mean, death around the time of birth. Stillbirth is oftentimes a misnomer in that many of us depend on our farmer friend for information in this regard. Was the farmer there to ascertain that the animal was dead on arrival, or did it die within a few minutes or a few hours before he made his observation? Therefore, if it lived 3 minutes or 3 hours it was not a stillbirth, but, as I see it, a perinatal death. This would conform with the nomenclature our physicians are using. I am sure these people know more specifically whether the babies are actually stillbirths or whether they die 3 hours hence.

Dr. Ray:

I would like to ask how long these infected animals remain infected if they don't contact more exposure? How long would a pig or cow be infected, once they were infected?

Dr. Stalheim:

I think Dr. Mitchell is here; perhaps he would elaborate on this. Dr. Mitchell has shown that swine may harbor leptospira for 2 years or a little longer.

Dr. Mitchell:

This was a very small group of experiments in 1930. We found great variation in the length of time we were able to demonstrate a leptospiremia. In a majority of them we were unable to demonstrate leptospiremia for longer than 6 months. The majority of the animals were kept for 26 months, and we were still able to culture leptospira from a few of the animals at slaughter. To some extent we felt we were able to correlate the incidence of leptospiruria with the serum titer in these animals.

Dr. Beveridge:

Could we hear some more about the nature of vaccines? It seems that Tompson in particular, for example, found vaccines quite harmless when injected in sows, and also that he did not establish infection. Is this correct?

Dr. Stalheim:

Yes, that is correct. I have manuscripts in the process of publication relating that vaccine did not cause infection in the kidney nor cause nephritis. And, that they adequately protected against the clinical forms of the disease, abortion, and renal infection.

Dr. Twiehaus:

Are you able to maintain the viability of the organisms for a good length of time so that it would be a practical vaccine? How do you maintain viability of the organisms?

Dr. Stalheim:

That is a very good question. I haven't been very successful with freezing as many other people have; but, it is a little surprising that you can leave a culture of leptospira on the table surface at room temperature and the culture will maintain itself for months. There is a drop quite rapidly, but after that, quite a little while. I am in the process of getting that straightened out.

Dr. LaSalle:

Have you made an attempt to back passage the antigen to make sure that it would not revert to virulence after you put it into animals?

Dr. Stalheim:

I have tried back passages in hamsters, three blind passages, and it did not become virulent. I have not been able to blind passage it in other animals. I have tried back passages in cattle; but, so far, it seems like the antigen disappears very rapidly in the blood, and I have not been successful.

Dr. LaSalle:

You said that swine may shed leptospira without detectable serum agglutinins. Could you tell how often this happens? What percent of the shedders are expected?

Dr. Stalheim:

No, I could not. I think it is rare.

Dr. Dunne:

I am very interested in the number of resorbing fetuses that were absorbed in cases that you have described that were associated with this infection. Do you feel this is a direct action of the organisms or is this an additional toxin effect? I am surprised that more of them are not expelled. In most bacterial infections, fetuses expel rather than mummify. Mummification is more common in virus infections.

Dr. Stalheim:

Yes, I haven't made comments along this line. Possibly pathologists would be more apt to be able to explain it than I.

Dr. Dunne:

I have personally used this as a rule of thumb criteria upon which to base whether or not we are working with a bacterial infection or a viral infection. When we come upon a case in which we are getting abortions, we think almost immediately of bacterial infections. When we see mummified fetuses at term, then we think more quickly of viral infections. I was very curious to know if this is a toxic reaction. Then I could understand that the organism is not pentrating the uterus and infecting the fetus and I could understand death caused by a toxic effect that was not strong enough to cause expulsion. I think the idea of mass expulsion will be demonstrated a little later and that mass is not the only criteria involved. Certain infections, like the hog cholera virus infection for example, will go to term and you will have everything dead. In some cases you will have one or two live pigs perhaps, but you can have all the rest of them dead yet the sow will not abort. So, I don't think it is entirely a matter of mass. I think it has to be something that is associated with a toxic factor within the uterus and then the uterus does not resolve and wants to expel.

Dr. Stalheim:

I would like to address to question to Dr. Twiehaus, concerning the numbers of stillborn pigs he has found in S.P.F. herds. Has he found any differences with regard to perinatal deaths?

Dr. Twiehaus:

I would say yes. We have a little incidence in our S.P.F. herds and some in our commercial heards. I would have to go through our records to dig this information up, but I would be glad to do this for you. Dr. Stalheim, I have question I would like to ask also. How many passages do you have to pass leptospira through before it becomes avirulent?

Dr. Stalheim:

Well, I don't know exactly. It is not easy to pass leptospira in synthetic medium. I go through a more or less witchcraft-type operation until I get better growth. When I do get good growth incidentally, there are variables among these.

Dr. Dunne:

You would believe though that repeated freezing of the leptospira organism would kill it?

Dr. Stalheim:

Well, it does in many situations. It doesn't in a high concentration of liver tissue.

Unidentified:

I would like to speculate just a minute on the reasons why these continual leptospiremias or concentrations in the ureters go on in the face of good circulating antibody. Plus the fact--why in vaccinated animals, one of the criteria that you use which is the prevention of renal leptospirosis, does the vaccine often look bad in this connotation, although it functions well otherwise. I think one of the guesses that we could make in this system is the place where the leptospira would be harbored in the kidney. Now the sections that I have looked at in the present materials, the larger amount would have a chronically infected animal with leptospira in the proximal convoluted tubules. When the organism is sequestered in this spot, it is essentially protected from the normal new methods of the animal. The antibody content of any exudate at this point is extremely low. So the organism is simply hiding from the new mechanism of the animal. In the reverse state, this also prevents antigenic stimulation of the animal. I think this is the reason we occasionally see these animals that have a leptospiruria without any measurable agglutins with the normal test. And, I think this is the reason the vaccines will often fail at this point. They will fail to prevent the establishment of renal leptospirosis, although they may work very well with every other criteria. It is so fine that you have a certain percent of your animals after vaccination which will still show signs of renal leptospirosis.

Dr. Dunne:

Dr. Stalheim, I would like to ask one other question. You referred to the common antigen not detecting this organism. Would you say that the commercial antigen would not detect these antibodies in any of these animals?

Dr. Stalheim:

Oh no, I don't think so. They have been carefully selected so they will get the most likely ones in this country, and those that are most likely to be introduced. It would be rather unusual, but possible that some other serotype not included in that pool could be introduced and these would not be detected.

Paper No. 8

SWINE BRUCELLOSIS

By B. L. Deyoe[1] and C. A. Manthei[1]

Brucellosis has been recognized as a major cause of abortion is swine (2, 29) since March 1914, when Traum isolated the organism now known as Brucella suis from an aborted swine fetus (32). Although there have been periodic major efforts toward determining the nature and means of eliminating brucellosis in swine on the part of persons in research, regulatory, and public health organizations, the disease is still important in swine production. In keeping with the topic of this symposium, this presentation will deal mainly with the role of brucellosis in reproductive failure in swine. However, other closely related aspects of swine brucellosis should be covered briefly as background information.

Brucella suis is the only Brucella species known to cause abortion in swine (27). There have been no confirmed natural cases of abortion in swine caused by B. abortus, although his species may infect swine and localize in lymph nodes of the head (21). Isolation of B. melitensis from tissues of naturally infected swine has been reported. Organisms initially misidentified as B. melitensis of swine origin in the midwestern United States are B. suis, type 3. Three biotypes of B. suis (types 1, 2, and 3) cause abortion in swine. Type 1 occurs in all areas of the world. Type 2 appears to be restricted to western and central Europe, while type 3 has been found in the United States, Asia, Africa, and South America (27).

Swine of either sex and all ages are susceptible to B. suis (15, 24). The disease is spread mainly by animal-to-animal contact, usually through ingestion or copulation. The oral route of exposure is probably the most common (23, 24), but during epizootics or enzootics the boar may be the chief spreader of infection through veneral transmission (3, 30). Brucellae are shed chiefly through semen, vaginal discharges, urine, or milk of infected swine.

After exposure to B. suis, swine usually develop bacteremia which persists for variable lengths of time (5, 15, 26). Thereafter, this generalized infection often develops into a disease of a localized nature. Numerous tissues, but most frequently lymph nodes, harbor B. suis (6, 15). The majority of infected swine are asymptomatic and at necropsy have no gross lesions indicative of the disease. It has been estimated that the disease is self-limiting in approximately 75 percent of the swine (26).

Clinical signs such as abortion, birth of dead or weak pigs, or sterility in sows and orchitis, lack of libido, or sterility in boars, as well as an occasional lameness or posterior paralysis in either sex may be helpful in the diagnosis of swine brucellosis. The most practical diagnostic method at present is the use of the standard brucella seroagglutination tests on a herd basis. The only confirmation of the disease is through isolation and identification of the Brucella organisms.

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Treatments have not proven to be effective in curing swine brucellosis (24). Furthermore, immunization procedures that are safe, practical, and effective have not been developed (15, 17, 24, 26). However, effective control measures are available and the disease can be eliminated by applying recommended methods for swine brucellosis eradication (31).

Reliable estimates of the incidence of swine brucellosis in the United States have been lacking. A statistically based survey of sows and boars at markets in 1966, using a modified seroagglutination test (Card test), indicated that 0.42 percent of the breeding swine in the United States were infected.[2]

Discussion of reproductive failure associated with swine brucellosis will be based upon observations recorded in the literature as well as unpublished data developed by the authors.

As to the latter, we have exposed 184 pregnant sows or gilts to virulent strains of B. suis, types 1 or 3. They were exposed by both natural routes (intravaginal, intracervical, breeding to boars that were shedding B. suis in their semen, conjunctival, or oral) and parenteral routes (intravenous or intradermal). Artificial and natural exposures via the genital tract were all done on the day of breeding, while those exposed by other routes were done at various times during gestation. However, 75 percent of the latter group were exposed between the 40th and 70th days of gestation. The establishment of infection was determined by one or more of three criteria: Persistent brucellemia, isolation of B. suis at the termination of pregnancy, or isolation of B. suis at necropsy. Susceptibility was not affected by the stage of gestation at exposure.

Abortions were considered as being caused by brucellosis only when B. suis was recovered from the blood stream, uterine fluids, placentas, colostrum, or fetuses. Abortions that occurred from 17 to 35 days of gestation were classified as early abortions. It was necessary to maintain 24-hour-a-day surveillance of these sows to detect many of the early abortions.

Embyronic or Fetal Death and Abnormalities

There is no recorded evidence to incriminate B. suis as a cause of embryonic death in swine. Reports containing the number of pigs per litter born to infected and noninfected swine (mostly serologic evidence) failed to show differences in this regard (4, 10, 12, 13). In fact, the reported average number of pigs per litter was frequently higher in sows with serologic evidence of brucellosis.

In our experience, exposure to B. suis via the genital tract does not appear to interfere with conception or to reduce the litter size. When gilts were exposed by breeding to infected boars or by intravaginal instillation of B. suis organisms immediately after breeding, 39 of 45 conceived at the first service. In an experiment in which gilts were exposed to B. suis by intracervical instillation immediately after breeding, 11 of the 15 principals conceived; while three of four unexposed control gilts conceived with one service. In the latter experiment, the average number of fetuses per gilt at 21 days or longer after breeding was 9.0 for the infected gilts and 8.3 for the unexposed controls. If B. suis was a factor in the cause of embryonic deaths, one would expect reduction in the number of pigs produced or in the conception rate.

Only Warnick and coworkers (33) suggested that embryonic death results from Brucella infection. However, they presented no reliable evidence that the swine in their study had brucellosis.

Although most fetuses are not viable when aborted, the majority of the available evidence indicates that circumstances leading to the abortion are initiated by severe metritis and placentitis rather than fetal death per se. There is, however, no conclusive proof that B. suis does not have a peracute lethal effect on the fetuses.

We have nearly always recovered B. suis from one or more aborted fetuses of each litter, but seldon from pigs farrowed normally by an infected sow (table 1). Brucellae were not recovered from any of 52 fetuses taken from uteri of pregnant infected sows at necropsy. B. suis was recovered from uterine fluids of 16 sows when no brucellae were isolated from their fetuses or

[2] Personal communication.

Table 1.--Recovery of Brucella suis from fetuses and uterine fluids of infected sows at the termination of pregnancy or at necropsy during pregnancy

Pregnancy	Recovery from --			
	Fetuses or newborn pigs[1]		Uterine fluids	
	Litters positive	Litters examined	Sows positive	Sows examined
	Number	Number	Number	Number
Outcome of pregnancy:				
Abortion	46	49	63	69
Stillborn or weak	1	3	3	3
Normal parturition	5	77	12	77
Pregnant at necropsy	0	13	5	8

[1] Considered as positive when B. suis was isolated from one or more fetuses of a litter.

pigs. In only one instance was B. suis recovered from fetuses of an infected sow without simultaneous recovery from uterine fluids. Histologic examination of aborted fetuses has not revealed pathologic changes in their liver, lungs, spleens, kidneys or hearts even though B. suis, type 1 was isolated from all of those organs. Acute to subacute purulent placentitis was observed. however.

Similarly, Johnson and Huddleson (19) examined 109 weak or stillborn pigs and six aborted fetuses, and recovered B. suis only from the aborted fetuses. Thomsen (30) found that B. suis, type 2 could be isolated from various organs of aborted fetuses, but most often from stomach contents.

There is no evidence from our observations or in the literature to indicate that swine brucellosis is associated with anatomic abnormalities of fetuses.

Abortion

Infected sows or gilts often do not abort. Those exposed to B. suis as suckling pigs (25) or several weeks before breeding (23) rarely abort. Hutchings (14) theorized that such nonpregnant swine pass through the acute stage of the disease and develop a resistance that prevents abortion, although they are still infected. Most of the evidence suggests that repeated abortions are infrequent. Only investigators at the University of Illinois (1, 8) indicated that a high proportion (9 of 32) of sows abort more than once.

McNutt (22) found uterine infection as frequently in nonpregnant as in pregnant swine and concluded that uterine infection was independent of pregnancy. We have obtained similar results with gilts that were exposed via the genital tract. Sexually immature gilts exposed by the conjunctival route did not develop uterine infection (5), but five of 12 ubred, 8-month-old gilts had infection in their genital tracts 60 days after conjunctival exposure. Therefore, uterine infection can occur frequently in sexually mature female swine, regardless of their status of pregnancy.

In field investigations of naturally occurring swine brucellosis, the incidence of abortion varied from none to near 100 percent (1, 8, 9, 13, 18, 19, 30). Only two reports (8, 30) stated rates of abortion greater than 30 percent. Murray and others (28) found that in 21 swine herds with serologic evidence of brucellosis, abortion had occurred in eight herds. The time of abortion in naturally infected herds has been reported to range from 21 to 105 days of gestation with average time ranging from 65 to 72 days of gestation. (1, 9, 11, 30).

In our experimental work, 73 of the 184 sows or gilts exposed to B. suis aborted because of brucellosis. Six additional abortions occurred which could not be attributed to B. suis infection. The incidence of abortion was highest in swine exposed via the genital tract at the time of breeding (table 2). Intravenous and intradermal exposure also produced high abortion rates, but these are not natural routes of transmission.

Table 2.--Rate and time of abortions in pregnant gilts or sows experimentally exposed to Brucella suis

Item	Exposure Method[1]			Total
	Intravaginal (27) Intracervical (11) Infected boar (16)	Conjunctival (76) Oral (11)	Intravenous (33) Intradermal (10)	
Number exposed	54	87	43	184
Number infected	46	70	39	155
Abortions, Number	29	23	21	73
Abortions, percent of infected	63	33	54	47
Early abortions, number	17	0	0	17
Litters stillborn or weak, number	0	2	1	3
Time of the abortion:				
Days gestation, range	[2] 17-62	39-106	61-99	
Days gestation, mean	34.6±13.6	73.9±16.7	78.5±10.9	
Days postexposure, range	17-62	10-58	13-34	
Days postexposure, mean	34.6±13.6	32.8±12.4	20.3±7.2	

[1] Figures in parentheses indicate number of pregnant gilts or sows exposed.
[2] Mean ± sample standard deviation.

Abortions occurred between 17 and 109 days of gestation. The time of abortion was more closely related to the time of exposure than to the stage of gestation, as evidenced by the decreased variability in days postexposure compared to days of gestation. With natural routes of exposure (genital tract, conjunctival, oral) the mean times postexposure when abortions occurred were quite similar regardless of the stage of gestation at exposure.

Early abortions occurred frequently when sows were exposed at the time of breeding. Under most conditions these abortions could have gone unobserved and when estrus reappeared it could have been assumed that they had not conceived.

Frequently, there were no warning signs of impending abortion, particularly in early abortions. Occasionally, sows had a hemorrhagic, purulent vaginal discharge for 12 to 24 hours before abortion occurred. On rare occasions the vaginal discharge was observed as long as 5 days before abortion. Following abortion or normal parturition, sows with uterine infection often had a visible vaginal discharge that persisted 4 to 6 days.

The expelled placentas were often edematous, some had focal ecchymotic hemorrhages, and some were covered with patches of exudate. The fetal fluids were usually reddish brown and often contained white to yellow flecks or granules. The fetuses appeared normal, although the peritoneal cavities of fetuses from a few litters were filled with serohemorrhagic fluid. Nevertheless, there was no gross evidence that would enable one to readily distinguish the aborted material caused by B. suis from abortion caused by other causes.

Occasionally, placentas are retained following abortion. Some sows also developed pyometra (23). Sows with these complications often develop fever, anorexia, weakness, depression, and rapid emaciation. The nongravid uterus often harbors B. suis, and the organisms may be shed for several weeks or months (23, 26).

Following abortion or prolonged uterine infection caused by B. suis, types 1 or 3, the most common gross pathologic change in the uterus has been diffuse catarrhal or purulent endometritis. Cystic endometritis occurred occasionally in sows infected with B. suis, type 1 (24). We have observed only two sows (one infected with type 1 and one with type 3) with multiple focal granulomatous endometritis. In contrast, Bendtsen and coworkers (3) and Thomsen (30) reported that 47 percent of the reacting sows they examined during enzootics of B. suis, type 2 infection in Denmark had the latter lesion, which they called "miliary uterine brucellosis." The frequency of this lesion appears to be an important difference between swine brucellosis caused by B. suis, type 2 in Europe and the disease caused by B. suis, types 1 and 3 in this country. Other differences between type 2 and types 1 or 3 are that cattle and humans have apparently never been naturally infected with B. suis, type 2.

Stillbirth and Neonatal Brucellosis

The birth of dead or weak pigs associated with brucellosis has been noted frequently (16, 18, 19). Data on the number of dead and living pigs born per litter in infected herds do not, however, indicate a remarkable difference between litters of serologically positive and negaive sows (10, 13). In our work (table 2), only three litters of weak or stillborn pigs were born to 155 infected sows (bacteriologic evidence). However, occasional weak or stillborn pigs also occur in many litters farrowed in brucellosis-free herds.

Neonatal brucellosis is not a significant problem in swine. Pigs suckling infected sows often become infected, but they remain asymptomatic and rarely develop bacteremia before 4 weeks of age (7). Furthermore, most of these pigs recover from infection before sexual maturity (7, 25).

Sterility

Apparent sterility may be the only manifestation of brucellosis in a swine herd. (4, 14, 15, 16, 19). Hutchings and Washko (18) found that 37 of 100 sows in one chronically infected herd failed to conceive. In a large herd studied by Cameron (4), 36 percent of 129 sows failed to farrow as expected. The following year, after brucellosis had been eliminated by replacement of the herd with their progeny, all the gilts farrowed normally.

The main cause of infertility in sows with brucellosis appears to be persistent metritis. Many sows that abort do not conceive readily thereafter. Hoerlein (12) found that if such sows were rested for two estrus periods before breeding, conception usually resulted.

In addition, a major cause of what appears to be infertility is the occurrence of early unobserved abortions caused by brucellosis. Sows that abort early usually return to estrus 4 to 8 weeks after breeding, and it is often assumed that they were infertile.

Conclusions

The etiologic agent of reproductive failure because of swine brucellosis in the United States is Brucella suis (type 1 or type 3). The role of brucellosis in such reproductive failure is as follows:

1. There is no evidence that embryonic death or fetal anatomic abnormalities result from B. suis infection in pregnant swine.
2. Abortion is the most serious complication of brucellosis in female swine. Abortion may occur at almost any time during the gestation period and is influenced more by the time of exposure than by the stage of gestation. The rate of abortion is highest in sows or gilts exposed via the genital tract at the time of breeding, and early unobserved abortions may occur frequently in such animals. The majority of pregnant swine with brucellosis do not abort. Also, Brucella-infected swine may abort from causes other than brucellosis.
3. Stillbirth or birth of weak pigs infrequently results from brucellosis.
4. Sterility can result from chronic endometritis, usually as a sequela of abortion caused by B. suis. The occurrence of early unobserved abortions can often be confused with infertility.

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DISCUSSION

Dr. Dunne:

Thank you, Dr. Deyoe. That is certainly an excellent coverage of a very important subject. Are there any questions anyone would like to ask Dr. Deyoe at this time? I know with all the problems of trying to get rid of brucessosis in cattle that brucellosis in swine should pose some problem. I think that as a public health hazard, certainly brucellosis should rank very high as a disease of animals.

MISCELLANEOUS ORGANISMS ASSOCIATED WITH ABORTION, AGALACTIA, MASTITIS, AND METRITIS

By H. W. Dunne[1]

In any symposium there is a need to include a miscellaneous section, usually referred to as "etc.," those items that generally are passed over lightly or ignored. If one were to search thoroughly, it is believed, however, that an impressive number of organisms associated with sporadic abortions would be detected over and above those already reported. Certainly, those organisms requiring special cultural techniques, media, atmospheric conditions, or specific host cells have not been adequately explored.

Furthermore, it is conceivable that all organisms need not infect the placenta or fetus or may not even need to be septicemic to cause fetal death and abortion. Most bacteria that do penetrate the placental barrier, however, produce either a purulent or a toxic reaction resulting in death of the placental tissue or of the fetus with their subsequent expulsion. Fetal death with retention and mummification is not usually associated with intrauterine bacterial infection, although this condition is common in virus infections.

Examinations of aborted fetuses by the commonly used laboratory bacteriological procedures have yielded largely negative results. More than 70 percent of the cases remain undiagnosed (12). However, a few organisms are recovered with some regularity thereby lending credence to their consideration as etiologic agents in swine abortion. Two of the agents, Escherichia coli and Streptococcus sp. also occur as fecal contaminants that casts considerable doubt, in many cases, as to their importance in the disease. There have been instances, however, when these organisms have been isolated in profusion and in pure culture, and it is difficult to treat their presence lightly. The difficulty is to know when the organisms are contaminates, when they are synergists or secondary invadors, and when they are the primary factors in the disease.

Little has been reported on the specific types of E. coli and streptococci involved. Generally, E. coli have been considered important only when they are hemolytic, although the validity of such judgment may be questioned. Streptococci, with both beta and alpha hemolysis, have been implicated although those with beta hemolysis appeared to be most important. Both E. coli and streptococci appear to be given more serious consideration when they are involved in early death of the piglet after birth. Both organisms, however, have been isolated in profuse pure cultures and are considered primary causes of abortion (9).

Other organisms isolated with some frequency from aborted fetuses in England are E. rhusiopathiae, Corynebacterium pyogenes and C. suis (11, 12). Salmonella also have been identified as a cause of abortion by Contini (2).

Staphylococci have been shown to be capable of causing an important abortion problem in Denmark (3). The organisms were apparently transmitted by the boar and caused abortion, metritis, and return to heat in 18 females. The lesions in the sows were those of a chronic disseminating endometritis. The boar had an actinomycoticlike lesion in the anterior part of the bladder from which Staphylococcus aureus was obtained. Two S. aureus types were isolated from the sows, differing in pigment, morphology, type of hemolysis, and some biochemical reactions but were of the same phage type and were coagulase negative. In another case, S. aureau was isolated in pure culture from various organs of 13 aborted fetuses (10).

Tuberculosis has also been reported as a cause of abortion in Ireland (5). Diagnosis was based on the presence of acid-fast organisms in the stomach of the aborted fetuses, calcareous

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lesions in the placenta, a positive avian tuberculin test in the sow, and the demonstration of small calcareous lesions of tuberculosis in the sow. Injection of the placenta into guinea pigs resulted in their giving positive reactions to tuberculin 26 days later.

Fungi also have found to be causes of abortion in swine, although this information is relatively sparse in the literature. Aspergillus fumigatus has been reported in England (3). Consideration should also be given to the aflatoxin-producing fungi and to Gibberella zea which is reputed to be a producer of an estrogenic substance. The ability of ergot to cause stillborn and weak piglets at term as well as agalactia will be treated under the following section head "agalactia."

In our quest to find the cause of abortions in animals, we must be aware, however, of the total complexity of the problem and try to find methods to evaluate the many contributing factors. The number of abortions obviously have been directly attributed to bacteria, fungi, or protozoa constitute less than 30 percent of the cases examined (12).

Agalactia--Mastitis--Metritis

The agalactia-mastitis-metritis syndrome is probably no less complex in the sow than in the cow. Losses resulting from the starvation of newborn pigs may range from 0 to 100 percent. Many attempts have been made to incriminate infectious organisms. From 70 cases of mastitis in sows, Cernea (1) isolated Streptococcus agalactiae from 36.1 percent, Strep. dysgalactiae from 10.6 percent, Strep. pyogenes from 23.4 percent, Staphylococcus sp. from 4.2 percent, and Escherichia coli from 2.1 percent. The disease was reproduced by infusion of the udders of 22 sows with Strep. agalactiae or Strep. dysgalactiae. Of these, 14 showed clinical symptoms of mastitis within 3 days.

Generally, no specific or uniform infection prevailed in the investigations of Ringarp (8), whose bacteriological examinations revealed a predominance of coliform bacteria. Indications were, however, that agalactic (or hypogalactia) was only one symptom of a many-sided symptom complex that characterized the disease (8). Blood analyses in agalactia toxemia frequently showed anemia, increased erythrocyte sedimentation rate, neutrophilic and eosinophilic leukopenia (with a possible leukocytosis later), and hypoglycemia.

Clinically, the first symptom observed was inappetence after farrowing, either complete or incomplete anorexia for one or all feeds (4). Temperature rise sometimes but not always was a feature. Vomiting, diarrhea, or constipation did not appear to be part of the clinical pattern. According to Ringarp who reported on 463 cases, 65.9 percent had diffuse swelling of all mammary glands. In 20.3 percent, a firm, sometimes sensitive, swelling occurred in one or more udder sections while other sections remained normal. In 7.8 percent, the udder appeared normal, with or without mild edema, whereas in 6.0 percent the udder was flacid with no symptoms of inflammation or evidence of lactation.

Symptoms of vaginal discharge, though often slight, were abnormal in more than half of the cases examined (8). The discharge varied from a thick mucopurulent to thin yellow liquid or pure pus. It is difficult to consider metritis as a separate disease entity because metritis, when it develops, frequently occurs at the same time agalactia appears. Metritis appears to be more of a disease syndrome involving both the uterus and the mammary gland to a more or less degree.

Treatment of the syndrome consisted of intramuscular injections of chlortetracycline, pituitrin, and in some cases predisolone. Generally, after one or two injections appetite returned and milk flow was restored. If the interval of time between onset and recovery was not too great, pig losses were kept at a minimum.

Marked agalactia was produced in sows fed grain contaminated with ergot (7). Characteristically, such sows had inadequately developed mammary glands. Only one-third of the pigs farrowed were born alive and these died, probably of starvation, within the first 24 hours. Such pigs were small and appeared weak at birth.

A relatively new approach to the syndrome was presented by Moore (6) who described a mastitis metritis condition from which he isolated a pleuropneumonialike organism (PPLO) to which he

give the name of Mycoplasma hyogenitalium. The organism was isolated from the uteri and udders of infected sows using a specially prepared medium. Four sows were infected with the organism. One sow, infected intravenously 20 days before term, died during farrowing. One sow exposed intranasally 5 days before term lost 11 of 11 pigs farrowed. Mycoplasma hyogenitalium was isolated by swabs of the uterine mucosa. Two other sows inoculated intravenously at 5 days before term raised five of 13 and six of 14 pigs farrowed.

Treatment appeared successful when 2 grams of Tylosin were given 2 to 3 days after farrowing. The addition of 1 gram of Tetracycline appeared to be better than Tylosin alone. Treatment alleviated the condition but did not eliminate the infection. Pretreatment of sows before farrowing reduced the clinical signs to a minimum.

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Dr. Stalheim:

After reading about the organisms in this area, would you get the impression that most of these organisms are now reduced by the antibiotics or did you go into this area of all?

Dr. Dunne:

I am certain that it enters into it, but I am glad you didn't ask me how much. I don't think we can really make any valid estimation on what is actually taking place. Certainly many of these organisms are becoming antibiotic resistant, but I am still not convinced that they alone are the key factor. I think that this one factor contributes, but like so many things, you can control one factor but not others.

Unidentified:

I am a practitioner, and I often see metritis and mastitis sows in my area. I always see this third day after farrowing. The first day the pigs will look normal. The second day, if you observe them closely, you begin to see a problem. The third day they are in trouble. For example, this fellow will farrow two thousand pigs a year. The first day everything was fine--the pigs looked normal, the mammary glands weren't hard, and there wasn't any discharge from the uterus. On the second day, there was a little discharge and the temperature just starts up. On the third day, you are in trouble.

You can treat these sows with anything, but if you don't use a cortisone, treatment is not effective. You have to have some cortisone, and the type product used doesn't seem to make any difference.

Dr. Dunne:

Would anyone else like to comment on this?

Unidentified:

We have a project on mastitis-metritis in progress, and we are as confused as anyone else. We would like to remind you of the paper that was presented at the AVMA meeting this last summer by the people of Purdue. They showed some evidence that there was possibly an endocrine discomfort in these too, where the ovaries were smaller than the normal sows and the mammary glands were poorly developed in the typical M.M.A. (Mastitis-Metritis-Agalactia) sows. I think we have to keep this endocrine discomfort very much alive here as part of the etiology in M.M.A. We have been trying to isolate mycoplasma, and we have not had any success at all. Is Dr. Simons here from Illinois? I think they have made a few isolations.

Unidentified:

He's not here. He couldn't make it. But he has made two isolants, but most of them have been bacteria, Staph and E. coli.

Unidentified:

That is what we have been coming up with. We have had absolutely no success with the other types of media, including Dr. Morris', and we haven't had the opportunity to compare his organisms with some of the one we are working with.

Dr. Beveridge:

We have the same problem, but we feel its a management problem.

Dr. Ray:

I just wanted to comment that this metritis-mastitis problem is not new. In the days when hogs were worth money, right after the first World War, I can remember Dr. Kinsley coming over into Illinois to see some of these purebred herds. He came home with smears, specimens, and whatnot. At that time we were getting Staphylococcus aureus and streptococci, and we used to make autogenous bacterins with these organisms. Sometimes this did a little good. We didn't have antibiotics, although we did have sanitary problems. I just wanted to add that it was not new. In the days when swine erysipelas was becoming established as a universal disease around the Midwest, it was not uncommon to find the first outbreak of the disease on the farm in sows at farrowing time. Some of the people had sows that died in the act of farrowing with acute erysipelas. Many of these sows would die and their pigs would live. The sow in the next pen would lose all her pigs and she would be sick and get well. But these pigs were dying with generalized erysipelas at 1, 2, and 3 days old. I haven't seen the condition in a good many years, but that is the way it started in the initial dissemination of the disease over the Midwest.

We never associated swine erysipelas before Dr. Van Ness considered erysipelas vaccine a completely safe product in pregnant sows and recommended its use to try to immunize them before they had their pigs so that they could get the colostrum immunity in their pigs. It wasn't considered a problem as far as abortions are concerned. We still have people who are diagnosing erysipelas arthritis in 10-day old pigs, but they've never cultured joint fluid and found erysipelas. I'll put in that way. This doesn't enter into infection in swine, but I thought I'd put these thoughts into the discussion.

Unidentified:

I would like to comment on this mastitis-metritis problem. I practice in central Illinois. In one herd in particular we were working with, Dr. Simons came and cultured the sows at farrowing time. Somewhere in the neighborhood of 120 cultures came up with nothing positive other than coli, the same staph and strep, with no correlation between the sows that had trouble and the cultures.

We have tried a little bit of everything on this farm, antibiotics at breeding time, mid-gestation, and antibody just before farrowing. In fact, to tell you how complicated this is, we had approximately 50 sows due to farrow and we broke this herd up into three groups. On one group we used high antibody; on one group we used a series of coli-straph-strep bacterin weekly, starting 3 weeks before they had pigs and continued it until they had pigs. On one group we used absolutely nothing--we used this group for a control. The results were that we had trouble with everything but our controls.

I was hoping you would have some answers today, but we are fighting the problem. I have been fighting for 6 years--the longer I go, the less I know. The only thing consistent on treatment we have used is Oxytocin.[1] We can use all kinds of antibiotics, but if we don't use Oxytocin we have a problem.

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[1]Affiliated, Armour-Baldwin, Burns, Corvel, Jen-Sal, National, and Osborn, containing 20 v.s.p. units of oxytocin for cc.

Unidentified:

Did you try an autogenous bacterin?

Unidentified:

Well, I tried this route too. In another herd in which we were having trouble, we cultured the organisms and had an autogenuous bacterin made. We used it on the herd and got absolutely no response. We had two bottles left, and I hated to throw it away. We had another farm approximately 30 miles away that was having the same problem. We used this autogenous bacterin on the other herd and got very good response. So this is the condition we find. We'd like to have an answer for it.

Dr. Beveridge:

We find that if we feed the sows bran, 5 days before farrowing, it greatly reduces incidences of this condition.

Dr. Dunne:

Well to me, one thing that is definite, it is an operation of the bacterial flora by virtue of putting a relatively inert substance into the intestine that does not support massive bacteria growth. Perhaps someone would wish to tie in the connection there. One thing I did want to comment on was that there does appear to be two slightly different types of situation. One type is one that develops about 3 days after the sow has farrowed, and the other is present when the sow farrows. In other words, the agalactia which is associated with the physiological upset, probably the feeding of pathogen contaminated feeds or something of this nature, is manifested by a failure of the sow to come to milk. And this certainly must be endocrine-related. The other is something that could very well be associated with the development of toxic products, either in the uterus or possibly in the intestine of the animal, as a result of failure to evacuate a buildup of dead body products that could certainly influence the animal. This is kind of discouraging, gentlemen, but nevertheless the problem does approach us. I see at the moment two different problems.

Dr. Kernkamp:

I think you might call this a secondary agalactia. If you have a litter of pigs that are sick and don't nurse, it is a matter of only a relatively short time when their mother will reduce her milk supply. Then, if the pigs recover, they are going to be a little short on food. This is a physiological thing.

Unidentified:

One other comment I'd like to make. The organisms that we have isolated I put in the category of the stress organisms, the ones that are involved any time conditions aren't right. But yet on the overall practice it seem like the herds that are having the best management have the biggest problem, and the herds that have the poorest management have the least problem.

Dr. Dunne:

Do these men generally reduce the feed before farrowing?

Unidentified:

Well, I can't answer that. I'm not sure. I find most of these sows never go off feed. The third day they are still eating good. The first and second day they're eating. The food consumption is right up and there is no constipation in these sows either. If you do have a constipated sow then you will have trouble. A lot of these third day metritis-mastitis sows will have a little period of discomfort.

Dr. Dunne:

The thing we don't know about the sow is what happens to all the body fluids during the pre-period of farrowing. We don't know anything about what happens to the intestinal contents during this period, let alone what happens in some of the other areas.

Unidentified:

Another comment that might be of interest. In the herds I am working the closest with, we have seen a discharge in gilts before they have been bred the first time. I believe this is a long duration thing. I don't believe it is something that occurs just at farrowing time or at any certain stage of gestation. It is my belief that it is there before they are even bred.

Dr. England:

I am not a veterinarian, so my remarks will be primarily on what is involved in the management of a swine herd. We've seen most of the things the gentlemen here have been talking about including the apparent worst infection in those herds confined and kept to a very high degree of what we normally think of as good sanitation. We've seen the puss discharge before the animals were bred both in the gilts and the sows. And we have seen where at times the udders would already be quite mastitic before the sows farrowed. Our veterinary diagnostic laboratory almost uniformily comes up with E. coli as either the major or only organisms it isolates. This has always shown to be susceptible to Furacin[2]. Our standard treatment has become to introduce about 60 to 90 cc. Furacin into the reproductive tract, with use of syringe and artificial insemination tube or pipette. Our control has been quite good, not perfect. We do still occasionally have some problems, but almost invariably this, along with use of Oxytocin for a day or so, has enabled us to avoid severe losses in the pigs. Now we have producers that use this with varying degrees of success; but I don't know how well they have used it. Our herdsmen just have to be keen on knowing when the sow needs the second treatment. I wonder if anyone else here has used Furacin for treatment.

Unidentified:

We have used Furacin installation at Oklahoma State. It apparently has done some good there.

Unidentified:

I'm glad to hear Dr. Beveridge say something about the feed because we have what we think are some real good swine operators in central Indiana. They have followed the sanitation practices, moved into new facilities, and came out with a big outbreak of mastitis-metritis. They swear by this level of feeding approach. I am sure it is a holding action, but they reduce the feed intake close to farrowing time or shortly thereafter and cut it down to one-half to two-thirds of what they normally feed. This has helped them considerably. Being a nutritionist, I still don't have any explanation for it, but at least they think it helps them a lot.

[2] Eaton Laboratories.

THIRD SESSION: D.P. Gustafson,[1] Chairman

Paper No. 10

PATHOLOGICAL FINDINGS IN MATURE AND NEWBORN MYOCLONIC PIGS

By M. W. Stromberg and D. P. Gustafson [2]

The disease in mature and newborn myoclonic pigs has been identified by a variety of names such as shakes, trembles, and jumpy pig disease. Kernkamp (14) suggested that the disease be named Myoclonia congenita. The earliest report which has come to our attention is that of Scholler (22) who observed what appears to be the same disease in Germany. Other reports appeared later in the European literature (10, 17). More recent literature has been received by Stromberg and Kitchell (23).

Myoclonia congenita affects primarily the newborn pig and manifests itself in the form of tremor of limbs, head or entire body. The tremor is postural in the sense that it ceases in the recumbent animal. Severity of tremor is highly variable both within and between litters. Most affected animals undergo clinical recovery in a matter of a few weeks. Tremor may sometimes be noted in the apparently recovered animal during stresses of cold or excitement and also following the injection of epinephrine or histamine (24). Neonatal death loss in affected herds may be considerable, but average figures indicate such death losses are similar to that found in nonaffected herds (23).

Theories advanced for the cause of this condition include poor nutrition of the dam (2, 5), hereditary factors (12, 13, 21), muscle fiber abormality (18), neurotropic virus (1), virus infection in the dam (4, 9, 19), and use of modified hog cholera virus for vaccination of the dam (3). The only reasonably consistent thread of observation concerns the possible role of the boar in a nongenetic transmission of Myoclonia congenita (11, 16, 19, 23).

Reports of gross or microscopic abnormalities in affected pigs have been comparatively rare. Several have reported normal findings (7, 18, 21). Christensen and Christensen (2) found a marked absence of normal myelinization throughout the central nervous system in three of nine affected baby pigs, slight to moderate reduction of myelinization in lateral tracts of the spinal cord in five, and normal myelinization in one. Hanson (8), reporting on 46 autopsies, found myoclonic pigs to show edema, thinkening, and hemorrhage in the transverse sinus region of the cerebellar dura, and variable degrees of congestion and hemorrhage at sites such as brain, lymph nodes, liver, kidney, extrinsic ocular muscles, lung, spleen, and thymus. Microscopically there was a widespread but mild vasculitis involving mainly the smaller arteries. The lesions were inflammatory, degenerative, or occasionally proliferative in nature. No consistent pathology was demonstrable in the nervous tissue. Harding (9) found cerebellar hypoplasia in about 12 percent of 1,115 brains from field cases of congential tremor. The earlier European observers noted that the tails of some trembling pigs became necrotic near the base and were later sloughed. It is worth noting that this is still a common finding in some affected herds.

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Earlier neuropathological studies (8) of the nervous system had produced discouraging results but the possibility of isolating a lession continued to be intriguing. The opportunity presented itself when we acquired five myoclonic pigs and decided to raise them to maturity. These showed a rather characteristic gait and all five had severe tremor which gradually improved. All showed some evidence of tremor (especially when excited) until time of autopsy at about 2 years of age.

Brains and selected parts of spinal cord were carefully removed as quickly as possible following electrocution and desanguination. These were then fixed in 15 percent formalin. Each brain was divided midsagittally and one-half was divided transversely into one-half centimeter blocks. Following processing several sections were cut from each block (about 20 blocks for each brain) and stained for myelin (Weil's), cells (Nissl), and general observation (hematoxylin and eosin). A variety of other tissues (heart, liver, spleen, lymph nodes, lung, thymus, thyroid, pituitary, adrenal, pancreas, kidney, and dura mater) were also examined histologically. Tissues from a mature sow with no history of Myoclonia congenita served as a control. Nervous and other selected tissues (see above) from six myoclonic baby pigs of less than 1 week of age were processed and stained in a similar manner. Also, each segmental level of the spinal cord was examined in one of these baby pigs. Cajal's technique for astrocytes, Holme's silver impregnation and several other special stains were used but not on all animals. Tissues taken from two newborn normal pigs served as controls.

The mature myoclonic pigs were clinically healthy normal animals with the exception of one which showed some hindlimb muscle atrophy and when observed, the tremor in these pigs was very mild. The myoclonic baby pigs studied showed a marked tremor that was obvious whenever the pigs were in a standing position.

Despite the mild or absent clinical signs, the mature pigs showed the most clearly defined changes in the central nervous system. Round cell infiltration of adventitia and perivascular spaces (figs. 1, 2, 3) of a number of larger vessels was seen in the brains of all five mature myoclonic pigs. Such changes were most prominent in the brainstem and diminished in a caudal direction. In some areas the nuclei seemed to line up along vessels in a single layer reminiscent of the way plant aphilds line up along a succulent twig. In other areas massive cuffs were seen along some of the medium-size arteries. Columns of large pale nuclei (fig. 4) were often present along the outside of small arteries, arterioles, and sometimes along capillaries. Smaller concentrations of such perivascular cells were seen in normal brains also. Special staining indicated that most were probably astrocytes. In all five mature myoclonic pigs such cellular depositions along vessels were widespread in the brain but not common in the spinal cord. There was no predilection for gray or white matter.

In general, there was a marked congestion with a high percentage of white blood cells in vessels of the brain. Occasionally red blood cells lay outside of vessels and larger meningeal veins often contained hemosiderin within their walls. There was much evidence of subpial and perivascular edema.

Vascular changes seen in organs other than central nervous system were largely proliferative with the intima showing the most obvious change. Some arteries were partly and some completely occluded (figs. 5, 6). These changes were most prominent in the heart of all five mature pigs but also seen in thyroid, liver, lung, lymph nodes, kidney, and dura mater of the brain. Small inflammatory foci (fig. 7) occurred in the muscle of both atria and ventricles in one of the mature pigs.

Perivascular round cell infiltration was rare in brains of affected baby pigs but careful study revealed increased numbers of what were presumably astrocytes along many of the smaller vessels (fig. 8). These changes were clearly less than in the affected mature pigs. Congestion of brain vessels was prominent and many vessels contained large numbers of white blood cells (fig. 9). Scattered vascular changes were also evident (fig. 10).

Figure 1.--Perivascular infiltration of inflammatory cells. Ventral medulla, mature myoclonic pig. X 80. Hematoxylin-eosin (H & E) stain.

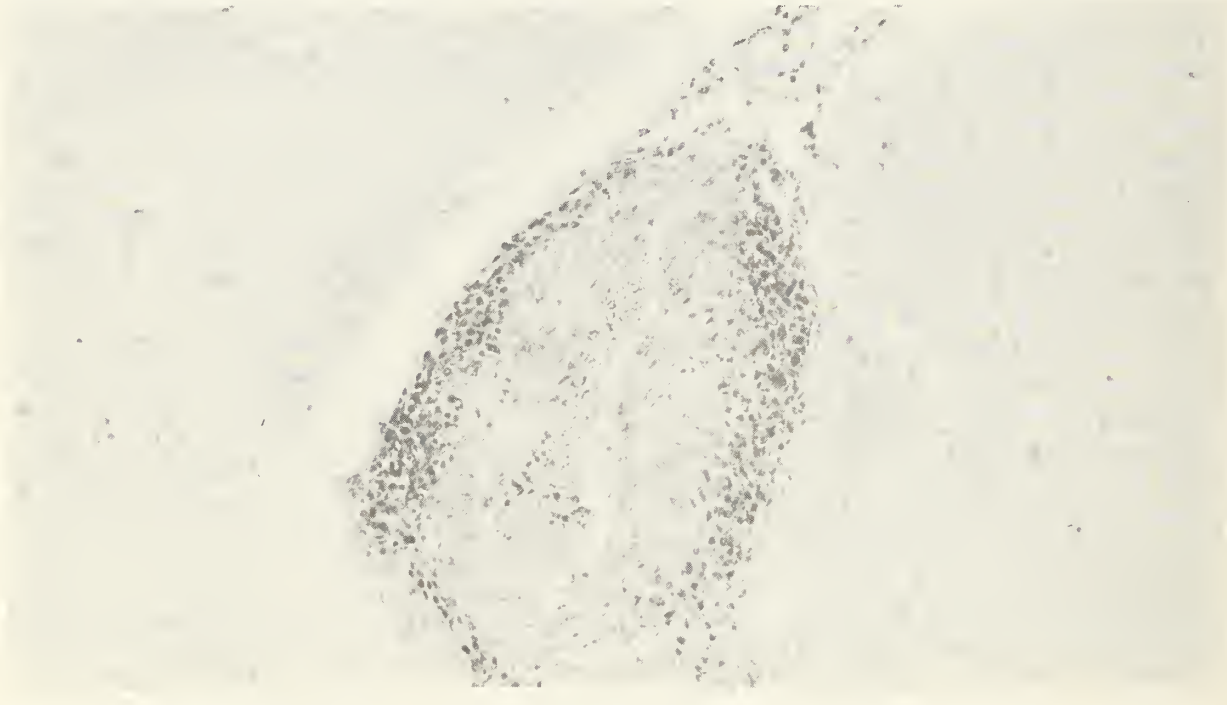

Figure 2.--Perivascular infiltration of inflammatory cells; some of these cells have invaded the media of the vessel. Thalamus, mature myoclonic pig. X 256. H & E stain.

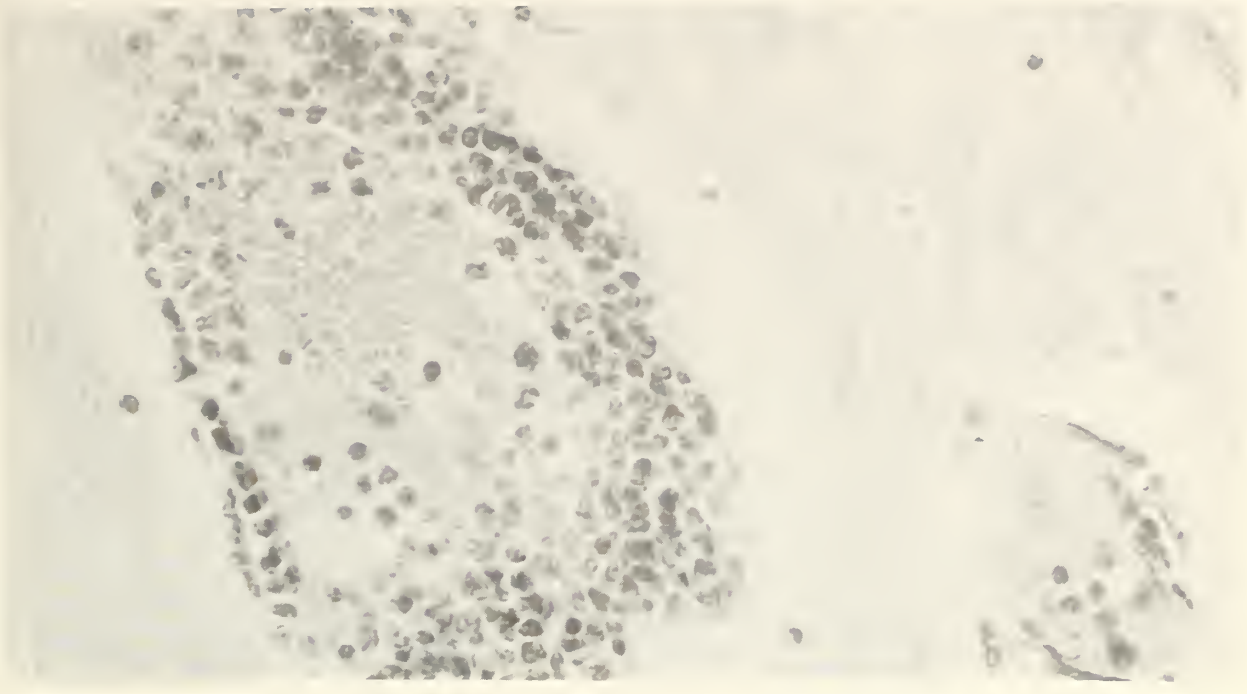

Figure 3.--Perivascular infiltration of inflammatory cells. Ventral pons, mature myoclonic pig. X 400. H & E stain.

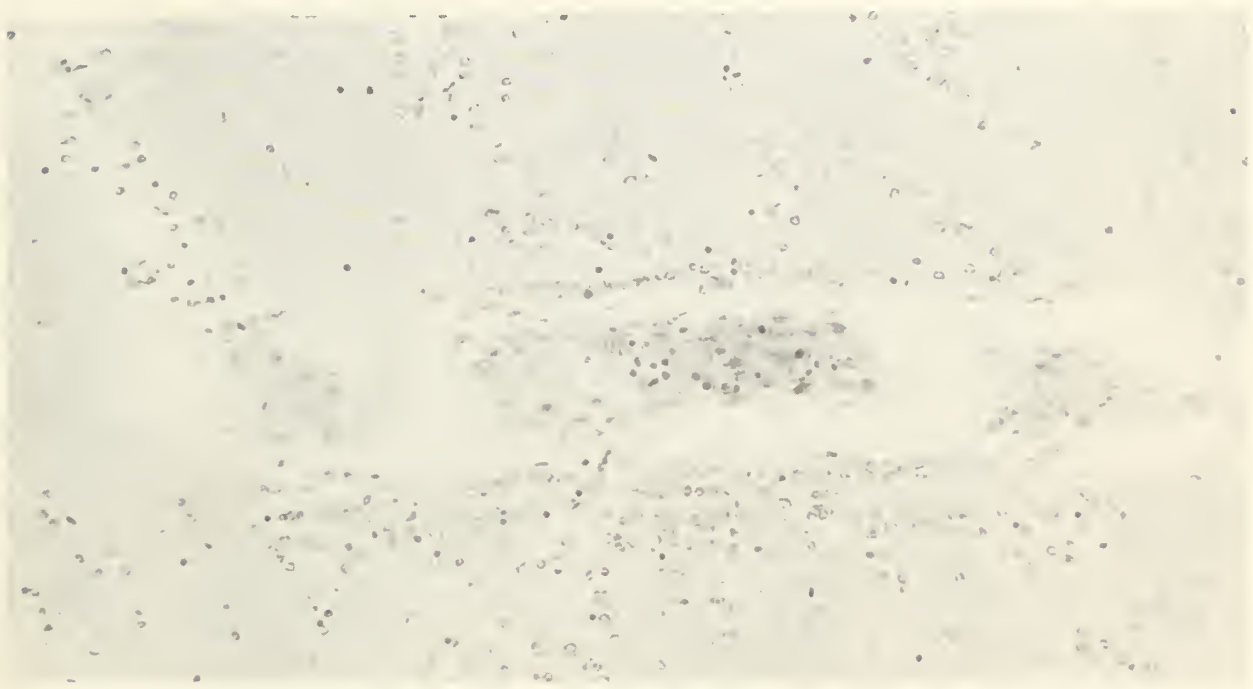

Figure 4.--Deposition of astrocyte nuclei along small vessel. Corpus callosum, mature myoclonic pig. X 400. H & E stain.

Figure 5.--Small occluded vessel. Atrium of heart, mature myoclonic pig. X 512. H & E stain.

Figure 6.--Small occluded vessels, scattered inflammatory cells, and many free red blood cells. Dura mater of brain, mature myoclonic pig. X 100. H & E stain.

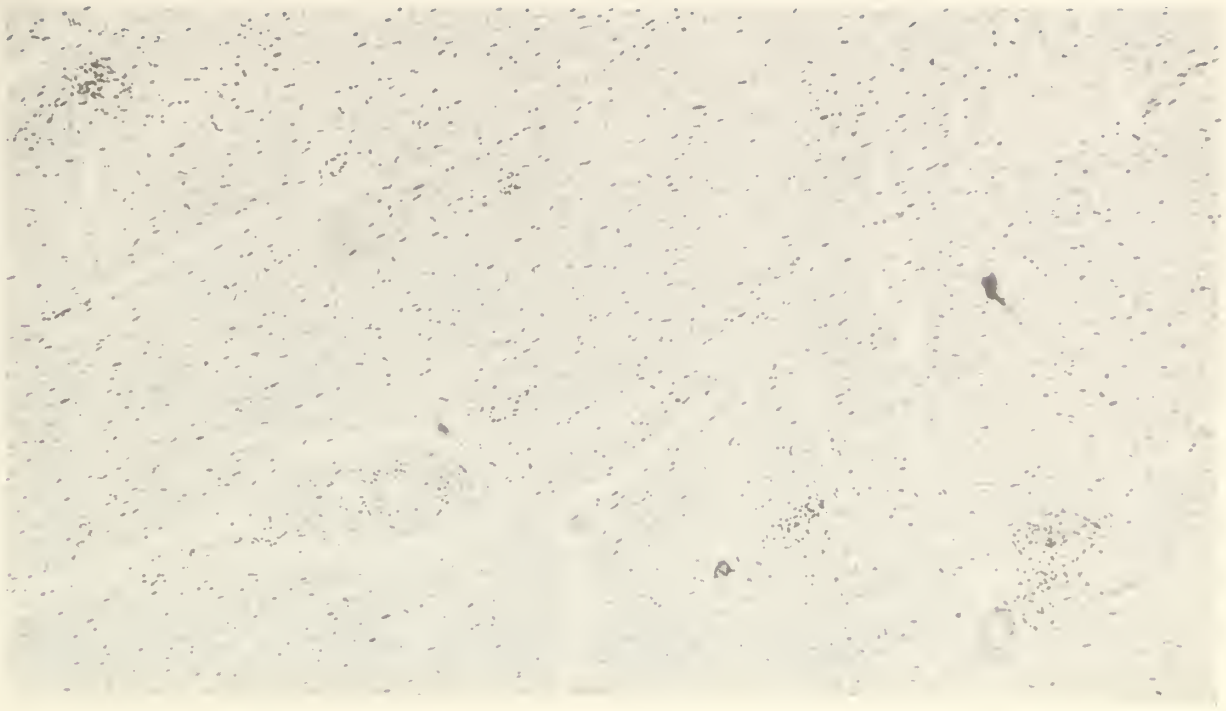

Figure 7.--Small foci of inflammatory cells in ventricular muscle of heart. Note irregularities in outline of intima in the blood vessel. Heart muscle, mature myoclonic pig. X 100. H & E stain.

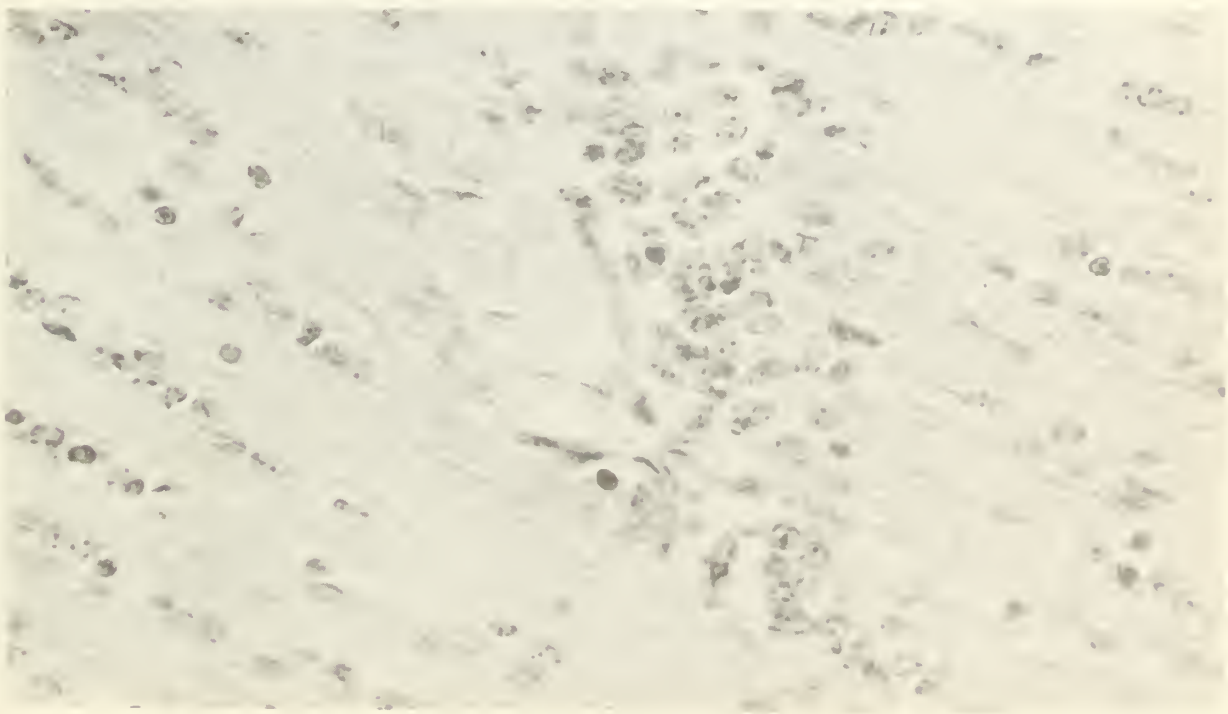

Figure 8.--Focus of large astrocyte nuclei. Optic tract, new-born myoclonic pig. X 512. H & E stain.

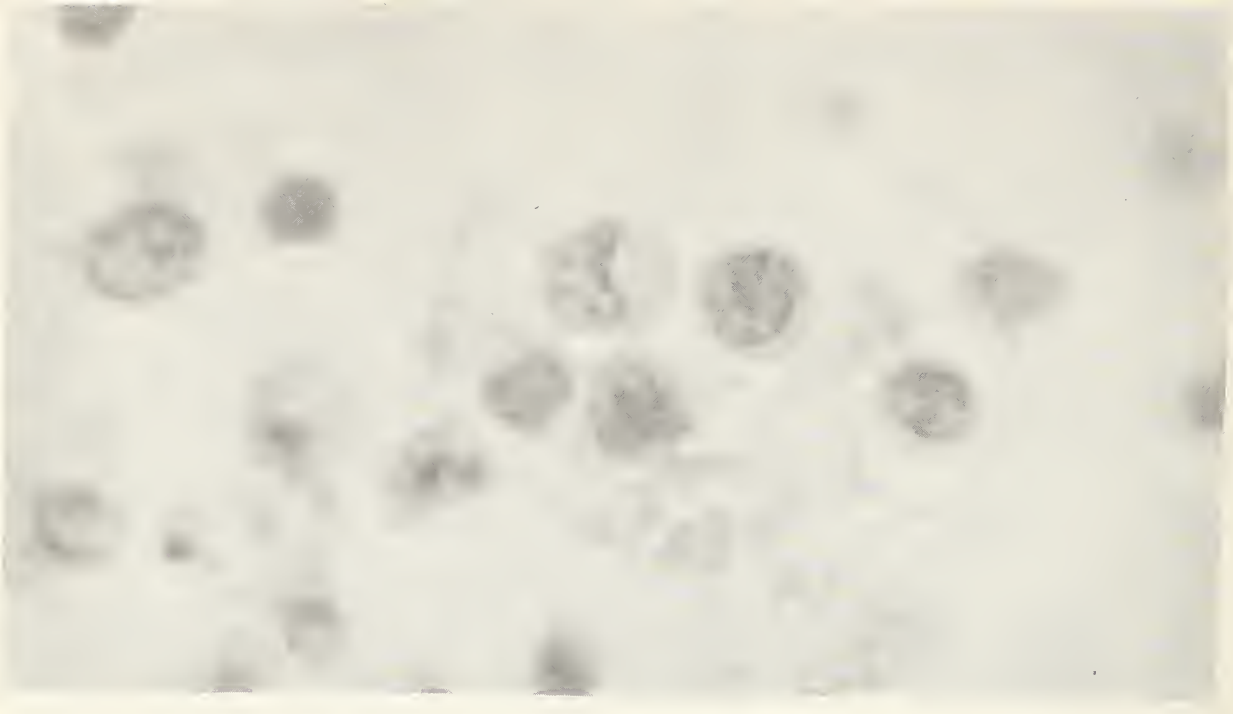

Figure 9.--Leucocytes. Small brain vessel, newborn myoclonic pig. X 2,000. H & E stain.

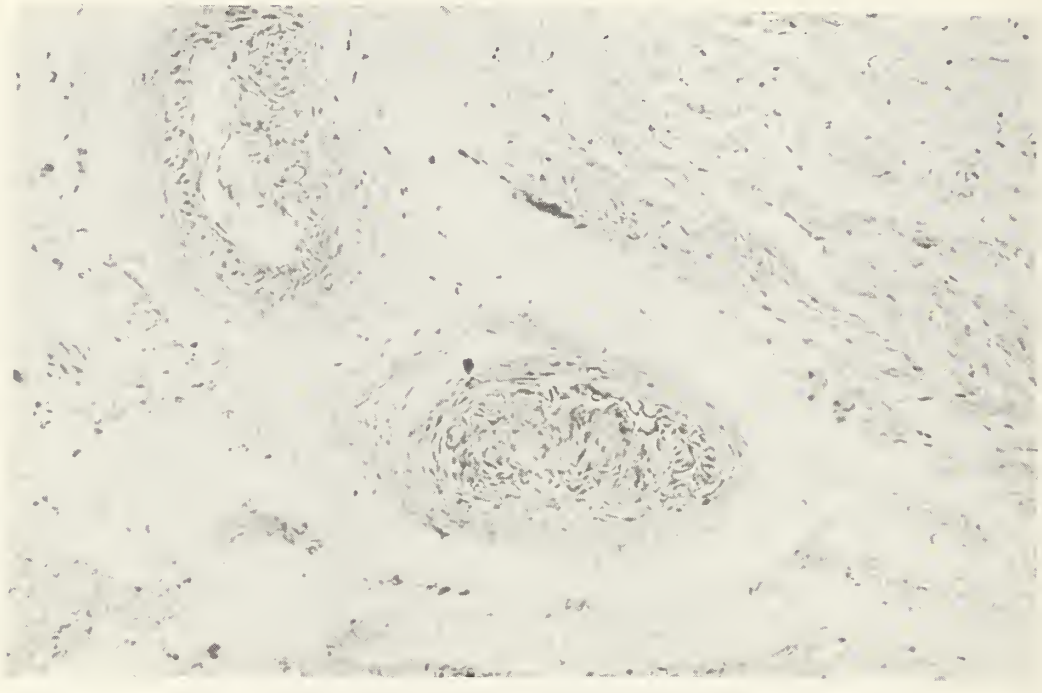

Figure 10.--Occluded vessel. Dura mater of brain, newborn myoclonic pig. X 160. H & E stain.

Evidence of neuronal destruction was generally slight but, nevertheless, rather widely distributed in the central nervous system (CNS) of both mature and newborn myoclonic pigs. Peculiar egg-shaped vacuoles were sometimes seen in the cytoplasm of both cerebral cortical and brainstem neurons. Rather severe vacuolization occurred in the lateral reticular nucleus and trigeminal motor nucleus of one of the mature pigs, but similar changes were detectable in the control brain also. Scattered neuronophagia was sometimes demonstrable in deeper layers of the cerebral cortex, the deep cerebellar nuclei and at various levels of the brainstem and spinal cord. There was no clear evidence of myelin destruction although it is possible that myelin formation was somewhat, retarded in the newborn myoclonic pigs. In some sections, areas of the cerebellum were seen to be nearly devoid of purkinje cells; but, since this was also true of control pigs, no significance was attached to it.

A peculiar structure (fig. 11) was noted to be plentiful in the brains of two newborn myoclonic pigs and later shown to be present in very small number in the other baby pigs both normal and myoclonic. These cell-like structures were usually spherical in shape, clearly outlined, and about 10 to 30 microns in diameter. With hematoxylin and eosin stain, these structures were usually a clear pink and contained two to 12 intensely basophilic, deoxyribonucleic acid positive, spherical bodies each of about 1 to 6 microns in diameter. At times these basophilic bodies appeared to be linked by fine filaments so as to create the effect of a small clump of grapes (fig. 12). There was no nucleus but at times the entire structure was seen closely applied to another nucleus in such a way as to suggest that it lay in the cytoplasm of the other cell (fig. 13). However, most of these structures were found lying free at various sites with the brain. There seemed to be some preference for the region around the lateral ventricles but they also occurred in the cerebral cortex and brainstem.

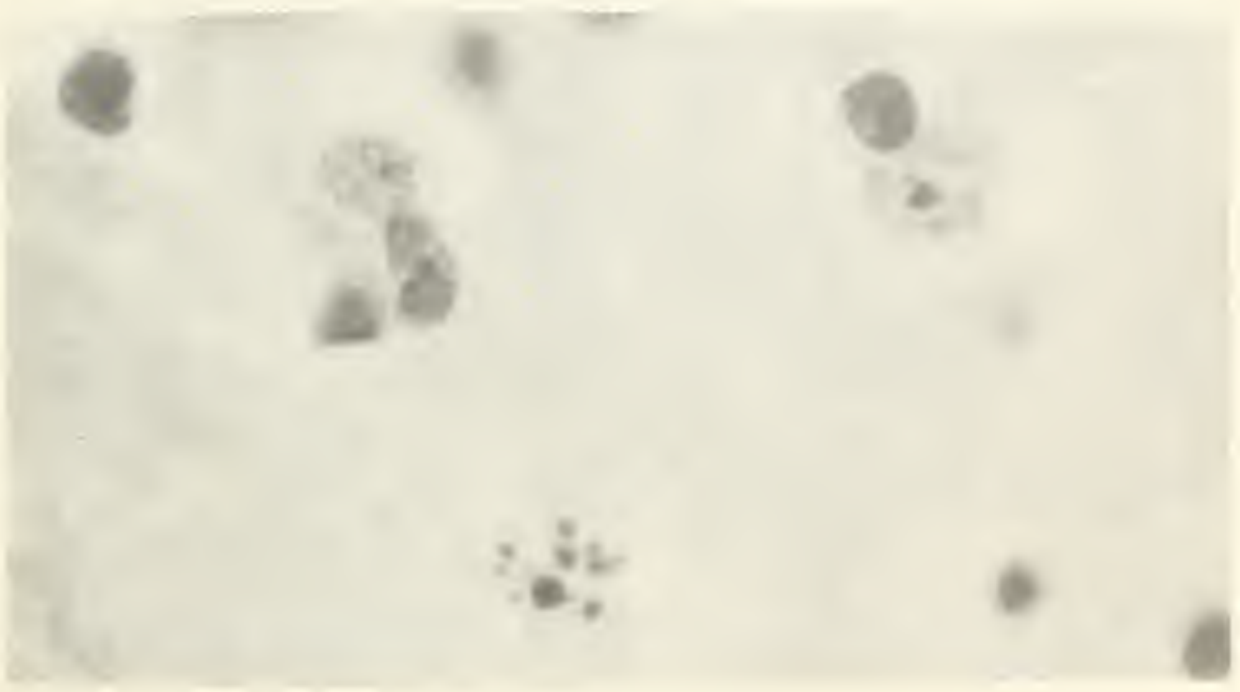

Figure 11.--Cell-like body with number basophilic particles. Brain, newborn myoclonic pig. X 2,000. H & E stain.

Figure 12.--Cell-like body with basophilic particles and filaments. Brain, newborn myoclonic pig. X 2,000. H & E stain.

Figure 13.--Basophilic particles adjacent to nucleus at top of photograph, newborn myoclonic pig. X 2,000. H & E stain.

Discussion and Conclusions

There is little doubt that Myoclonia congenita results from intrauterine developmental disturbances under-gone by the fetus. With rare exceptions the affected baby pig manifests a tremor immediately after it is born. Action of infectious or toxic agents early in gestation could be expected to result in disturbance of organ development of a recognizable nature. The absence of any prominent and consistent malformation (see Harding (9) for opposing view) in myoclonic baby pigs suggests an etiological agent which acts late in gestation. Since the most probable route by which such an agent can reach the fetus at that time is via the uterus it is reasonable to assume that the toxin or infectious agent is carried by the blood of the dam. Genetic factors appear to be ruled out by the results of consecutive matings (11, 16, 19) and the absence of tremor in offspring of matings of myoclonic pigs (7, 19).

The generally mild reaction in both vascular and CNS tissues of myoclonic baby pigs does not point clearly to either an infectious or a toxic cause. Neither is there apparently any consistent destruction of a specific region of the nervous system that might explain the tremor. Earlier works (25, 26) suggest increased lower motor neuron excitability in myoclonic pigs, but no morphological basis is evident.

Further confusion is added by the findings reported here on mature myoclonic pigs. The vascular inflammatory changes are suggestive of virus infection or possibly a hypersensitive response to an antigen of some sort. The most intriguing aspect would seem to lie in the possibility of studying long-term changes in brains of myoclonic pigs. We might anticipate further progressive deterioration which would later lead to clinical signs in affected pigs and perhaps serve as a model for the study of progressive degeneration of central nervous system in man.

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DISCUSSION

Dr. Twiehaus:

I was wondering if you observed any temperature in these five pigs that you maintained?

Dr. Stromberg:

We recorded temperatures for a month or so. They were always normal so we lost interest in this. We kept these animals for quite a long time. This particular episode from which we obtained these five large pigs came out of a farm where they have purebred animals. They had quite a seige on their farm during a farrowing period of a year or more. These five were

chosen from a lot of around 300 pigs. We could pick them out because they were smaller than the balance of the group. Also, when we would chase them up to one end of the lot, the ones that were affected would develop a peculiar gait after we chased them around for a while. It would have been remarkable if we would have been able to pick out five from the same litter. So they came from this kind of a group.

Dr. Dunne:

Inasmuch as there is evidence of accumulation of leukocytes within blood vessels, did you by any chance do any blood determination at the time of necropsy?

Dr. Stromberg:

No, not from these. However, we did a few of these, 8 or 10 years ago, which didn't come out normally. You don't see these increased numbers of leukocytes anywhere but in the brain; at least, I haven't. So I don't know whether the average number would be changed, but this could certainly be checked.

Unidentified:

Was there any evidence of any viral infection on this farm?

Unidentified:

Well, not that we know of. This fellow has an unusually excellent herd of swine, and they are given the usual vaccinations for hog cholera as well. They are all animals that have been raised on the farm.

Dr. Beveridge:

They have been doing quite a lot of work on this condition, and allied conditions at Weybridge. I hesitate to speak, I may be wrong in reporting what they have been doing; but, as I understand it, they have found that in quite a number of cases where this condition has occurred on farms there has been hog cholera on the farms. As you know, they have been stamping out hog cholera, and in some instances they have located farms where this condition existed. On further observation and testing, they have found out there has been hog cholera behind it. But, this is certainly not the explanation of all cases. They have been investigating this condition in pigs and allied conditions in lambs and in calves, which they term hypomyelogenesis congenita. In the case of the lambs, I understand that they have been successful. I think it has been just recently reported in the Veterinary Record that the condition can be transmitted by taking material from a newborn affected lamb and inoculating the ewe at various stages of pregnancy. I think similar experiments have been done in pigs, but I cannot give you the results. It does look as though there is quite a bit of evidence that there may be some, as yet not recognized virus, causing the condition in pigs perhaps and it seems certainly in lambs. Clinically when the pregnant ewes were inoculated I think it produced no disease. It seems possibly to be subclinical in the adult, but nevertheless, affect the young in utero. This work is just being developed and, as yet, I hesitate to say very much about it because I may not have the thing completely straight.

Dr. Stromberg:

I should have brought this out. As I recall they reported on quite a large number of myoclonic pigs. I think their figures were that about 12 percent of these showed the cerebellar hypoplasia and also a lack of normal myelinization. These, I think, were the ones that they associated with hog cholera. You are right in saying that it isn't at all clear.

Dr. Gustafson:

I visited with Dr. Done this spring, and we went over this in a conversation. The part of the myoclonia that was associated with hog cholera did show the cerebellar hypoplasia. He agrees that not all myoclonia can be associated with hog cholera. Furthermore, the myoclonia associated with hog cholera is associated with certain strains of hog cholera. He believes that the type of thing we have seen and we are trying to describe here is separate from hog cholera. At least, that was the gist of our conversation at that point.

Dr. Beveridge:

It seems likely, doesn't it, that perhaps there may be several neurotropic viruses affecting the developing animals that produce this type of condition?

Dr. Gustafson:

It may be that the kind of thing we are seeing expressed in pigs may be expressed differently in other animals as well.

Dr. McDaniel:

Would you care to comment as to whether you feel the vascular lesions that you are seeing are in fact caused by direct action of the virus or possibly to an allergic encephalitis?

Dr. Stromberg:

No. I would like to have Dr. Dunne's comment on that.

Dr. Dunne:

Strangely the virus infection of foals, periarthritis in foals, has a lesion that is strangely very much like you described and illustrated.

Dr. Stromberg:

I think both of these things are compatible. The conjecture has been that the diseases are one and the same but express themselves differently in each species. This thought has occurred to some who have interest in both diseases; however, at this time no one is going to say definitely one way or another.

Dr. McDaniel:

One other question. Has there been any serological studies to see if there were any antibodies against this encephalomyocarditis virus?

Dr. Stromberg:

When we took the brains from these animals, we didn't know what we were going to find. If we had some inkling on this, maybe we would have done something differently. This came as quite a surprise.

Dr. Twiehaus:

Did you observe any eosinophilic bodies or inclusions in any of these cells in the brains?

Dr. Stromberg:

Nothing.

Dr. Ray:

I can't help but add this comment. I have seen a lot of these shaker pigs. I've seen some shake their tails off. We have had this plague on certain purebred farms where pig after pig after pig would lose their tails. I have done everything I knew to find out what this infection might be. John Bryant at Mt. Vernon, Iowa, sent me a little sack one time that had 27 pigs' tails in it. It had been diagnosed as erysipelas. It wasn't erysipelas and it is not erysipelas; but, I still don't know what it is. We found out through the years when you run into this thing it gets to be a malady in a bunch of farrowed pigs. If they would take equal parts of iodine and glycerine, it was an effective treatment. Some of these farmers got so they just rode that pig's tail plumb up to the root of their tail all the way back. You stop it if you put the iodine-glycerine mixture on, but don't wait until it gets hard and then put it on.

Unidentified:

What was the hog cholera vaccination status on this farm?

Dr. Stromberg:

They had all been vaccinated. I believe they had all been vaccinated with some of Dr. Ray's vaccine.

Unidentified:

Has chorio-meningitis been ruled out of this?

Dr. Gustafson:

I believe so. These didn't have the cellular reaction that we would expect to find in that.

THE EFFECT OF PARASITES AS RELATED TO INTRAUTERINE LOSSES[1]

By William D. Lindquist[2]

There are several ways in which intrauterine losses of pigs can be accomplished by parasitism. The first type of loss and most striking of these is, of course, the death of the sow and loss of all in utero piglets. Parasites such as Trypanosoma simiae can cause such losses. A sow could be infected for 4 to 6 days, the prepatent period, according to Stephen and Gray (21), and die within another 3 days. Unfortunately, the extensive monograph by Stephen (22) does not include any information nor data, which probably is yet to be collected, concerning effects on pigs in utero, or in utero infection, abortions, or stillborn fetuses.

Abortion associated with trypanosoma infection is not unknown in other hosts. Levine (10) mentions this with T. theileri of cattle and T. evansi in camels. Morgan and Hawkins (13) indicated such an association with infections of T. equiperdum in horses. Although not recorded, it is not unlikely that such a phenomenon may occur with T. simiae in pigs. Fortunately T. simiae does not affect us in the United States but the author saw in West Africa, a fine herd of an imported breed of pigs, about 20 gilts and a boar, wiped out in a matter of a few days with this disease.

The second type of loss caused by intrauterine infections and one present in the United States is that of stillborn or premature births caused by Toxoplasma quondii. Cole and coworkers (5) in 1953 reported a 50-percent mortality of baby pigs on a farm with 138 deaths of piglets--some stillborn, some premature but the majority dying from 1 to 3 weeks of age. Toxoplasma organisms were found in the lung, bronchial lymph nodes, intestine, and heart. Mouse inoculations from these materials were positive for Toxoplasma. Three of the sows from the farm reacted positively to the intradermal test so baby pigs, placentae, and allantoic fluid were collected from the birth canal. Mouse inoculations were positive on tissues of several of the pigs, but no positive tests occurred from the placentae or allantoic fluid. This seems strange but may be a result of the small sample. The same authors determined by mouse inoculations, that milk from sows positive by skin test contained Toxoplasma. This phenomenon had already been demonstrated in canine Toxoplasma infections.

A third type of loss caused by intrauterine infection occurs sometime after the animal is born. Strongyloides ransomi and Stephanuris dentatus produce such losses.

It has taken us some time to accept the seriousness of prenatal infections of Strongyloides ransomi of pigs. Spindler in 1937 (17) mentioned finding immature threadworms from the bodies of pigs necropsied 3 to 11 days after birth. Andrews and Connelly (2) reported finding a gravid S. ransomi worm in a 4-day old pig. Neither of these authors postulated a prenatal infection, although Lucker (11) found the prepatent period of orally infected pigs to be 6 to 7 days. Spindler and Hill (18) in 1942 determined the cause of death of some young pigs (both earlier and later than 2 weeks of age) to accumulation of Strongyloides larvae in the heart. In 1943 Spindler and others (19) pointed out that pigs raised in unsanitary, naturally seeded pens failed to gain more than one-half of the weight of litter mate controls raised under sanitary conditions.

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[1]Published with the approval of the Director of Michigan Agricultural Experiment Station as Journal Article Number 4139.

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The first evidence of possible prenatal infections was published by Enigk (6). He commented: "Trans-placentar Strongyloides invasion occurs in pigs as proved by experiments." Enigk percutaneously infected a pregnant sow during the third and fourth month of gestation 20 times with 2,000 larvae each time. In the resultant litter of six pigs Strongyloides eggs could be found in rectally taken fecal samples of five animals 4 days after birth. The sow never showed a patent infection even 6 weeks after parturition.

Batte and others (4) infected newborn pigs by feeding colostrum from Strongyloides egg negative sows with a resultant 4-day prepatent period. Some pigs from the same group were fed either cow's colostrum or filtered sow's colostrum and did not become infected. He also demonstrated infective Strongyloides larvae in sow's colostrum before and 12 hours post parturition. There is a paradox here. Enigk (6) failed to find Strongyloides in one pig killed at birth and yet found eggs in the other five at 4 days of age. Was his infection a colostrum infection? On the other hand, Batte (4) noted, by oral colostrum infection, a prepatent period of 4 days while normal infections by Lucker (11) took 6 to 7 days. Is there something about colostrum Strongyloides that speeds up their development?

In 1963 Stewart and others (23) fed large numbers of S. ransomi larvae to a pregnant sow. Fecal samples from the sow remained negative. Of the six pigs farrowed one was stillborn (reason not given) and another killed immediately. A careful search for larvae recovered from the carcasses by tissue Baerman apparatus revealed them in the lungs and liver of both pigs but their intestines remained negative. The remaining pigs started passing eggs at 2, 3, and 4 days of age. Stone (24) found Strongyloides larvae in the muscles of a pig examined at birth when the sow had been experimentally exposed, orally and cutaneously, to a large number of infective larvae during a 2-month period before farrowing.

If one can pause to speculate here, Strongyloides infection in pigs may be following a recently discovered pattern of helminthiasis. For instance Sprent (20) and Webster (29) showed that canine bitches may acquire a latent Toxocara canis infection which becomes activated during pregnancy. The result is a prenatal infection of the puppies but often no patent infection appears in the bitches. It has also been shown by Olsen and Lyons (15) that Uncinaria lucasi, a hookworm of seals, is passed to the newborn not only by the usual cutaneous route but also by the colostrum of the cow.

One may summarize Strongyloides infections in pigs by saying prenatal infections occur but larvae develop only to a certain stage in the liver, lungs, and muscles but rapidly become patent in 2 to 4 days after birth. The triggering mechanism for further migration and development is unknown. Young piglets may also acquire postnatal infections from the colostrum of the sow.

Regardless of whether infection occurs by intrauterine or colostral route the main point to be made is that this parasite contributes to death of very young pigs. Batte and others (3) indicated losses on endemic farms as high as 75 percent before pigs are 2 weeks old. Riesinger (16), reporting on Strongyloides outbreaks in Austria, indicated a 50 percent mortality.

The other helminth-producing prenatal infections in pigs is Stephanurus dentatus, the swine kidney worm. For some years investigators failed to produce patent laboratory infections only because no one was forbearing enough to wait for a long period of time. Tromba (25) finally produced such infections in pigs by oral feeding of earthworms and by continuing his experiment as long as 240 days. Batte and others (3) produced patent infections in 9 months by oral earthworm feeding and in 16 months with orally instilled infective larvae. The disease was said to be patent only in older animals until Batte and coworkers (4), while visiting swine abbatoirs noted ureter cysts in pigs less than 5 months old and upon urine examination of some of these, found viable eggs present. Experiments were devised to check prenatal infections by infecting bred gilts and allowing farrowing in airlocked isolation rooms. These gilts never showed patent infections, but piglets either provided typical liver lesions and in some cases migrating larvae, or if held long enough showed larvae in the perirenal fat. Deaths that were encountered occurred early but with obscure causes.

There does not seem to be collected data of intrauterine losses with Stephanurus dentatus but losses in condemned livers are great in endemic areas and baby pigs may certainly get their infections in utero.

It is apparent that those parasites involved in intrauterine infections are ones that either live in the tissues or have migratory phases through tissues on the way to the intestine or kidney. For this reason, Ascaris suum has long been a suspect. Over the years the preponderance of evidence (Martin, 12; Van der Wall, 26; Alicata, 1; Kelly and Olson, 1; Olson and Gaafar, 14) and experiments in the author's laboratory have yielded negative results. However, Lee (8) while failing to produce experimental prenatal infections did find one larvae of an ascarid in the lung of one out of 48, 10-hour old piglets purchased in the market. In 1963 Lee (9) failed by several routes to infect pregnant rabbits with viable Ascaris eggs. Because of recent colostrum findings, this avenue should be explored before giving up on explanation of ascariasis in very young pigs.

Probably one of the best analysis of causes of mortality in pigs was presented in two papers (27, 28) by the Veterinary Investigation Service of the Ministry of Agriculture, Fisheries and Foods in Great Britain. Careful records were kept on the causes of death of all piglets in a sizable sample of herds. The preweaning losses according to age group were listed from 28,400 pigs. A quarter of the pigs born failed to reach 8 weeks of age. Fifteen percent of this quarter died in the first week and another 6 percent was stillborn. In their listed causes of death one finds: "enteritis nonbacterial" and "pneumonia." Could migrating Strongyloides, Ascaris, or Toxoplasma be responsible for either triggering or causing overt pneumonia? Could prenatal strongyloidiasis be responsible for nonbacterial enteritis? One realized that a pathologist can section only a limited amount of tissue, but does he also do intestinal mucosal scrapings in an effort to find Strongyloides? Fifteen to sixteen percent of the deaths in the first week had no observed cause. Could Strongyloides larvae in the heart or Toxoplasma in one of many of the body organs be involved?

The diagnosis of prepatent parasitism is not very advanced. Often during the migratory phases the damage is done and by the time patency occurs the animal has recovered or has already been culled. There are no immediate answers to this problem, but we can look harder for prepatent parasitism and in some cases utilize more tests to cut down the percentage of "death without cause."

Over 70 percent of absortions and stillborn piglets have defied our ability to determine etiology (27). This figure can and should be reduced with more careful examinations and use of more diagnostic techniques. In such cases the use of Baerman larval recoveries from the tissues, microscopic examinations of scraped gut mucosa, tissue press examinations, and more histopathological studies of heart, brain, and other organs would undoubtedly reveal more etiology. A share of this may well be intrauterine-acquired parasitism.

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DISCUSSION

Dr. Gustafson:

Thank you very much, Dr. Lindquist. That was a very, very interesting paper, and I am sure it must be stimulating to many of us who are working with losses in this regard. We are wondering the same things you are wondering about, the effect of parasitic infections on our experiments. This along with Joe Conrad's work are, what seems to me to be, relatively small margins of differences. This kind of thing could contribute to variations in our work. Are there any comments or questions from the floor concerning this paper? It seems to me that with the input of nutrition, along with the endocrinological features and the genetic factors that I think have been brought to mind today, and now parasitism, that things are indeed as was suggested when we started, quite complex. There probably are very good reasons for the large area of the unknown in this particular field of intrauterine losses of baby pigs.

Dr. Twiehaus:

I might comment, Dr. Gustafson, on work we have done in our specific pathogen-free (S.P.F.) pigs in regard to ascarid larvae. We have been particularly interested in the so-called blue liver that we see with mass ascarid larvae infection in swine. We have found that the reaction we observed in these pigs is apparently a sensitization lesion. If you take S.P.F. pigs and run ten thousand--fifty thousand--a hundred thousand ascarid larvae though them, the first time you will see no particular reaction in the liver. But the next time you expose these pigs you get the marked reaction of the liver. This response is rather dramatic and occurs in a matter of 5 to 10 days. This is when we get this particular reaction that we think is definitely a sensitization occurring in the liver that causes the so-called blue liver in the fetus.

We have done a little work with radiating some of these larvae and passing them. We can produce the same type of lesions when we radiate the larvae as are produced with the two passages of the nonradiated larvae. There is no particular explanation for this. As yet, we feel there is some disturbance in the metabolic pathways, or enzymes, in this particular aspect which is probably responsible for this. This is a very likely field for further investigation.

Dr. Lindquist:

I think this is a nice kind of experiment, when you can give a single charge of ascarids and look at the lesions and find nothing. However, it doesn't work that way on pastures because these pigs are getting daily charges, or maybe hourly, so that a pig on pasture is very quickly sensitized. When you kill these pigs, most of their livers have lesions.

Dr. Gustafson:

Dr. Waxler, I wonder if you would have any comments on gnotobiotic pigs from the standpoint of strongloides?

Dr. Waxler:

No, but I would like to ask Dr. Twiehaus a question about this secondary reaction, reaction to the second migration. Is there eosinophil response?

Dr. Twiehaus:

Yes, there is eosinophilic response; but it is primarily an edematous type of reaction. This is why we feel that it is more sensitization than it is inflammatory. These reactions will subside if you don't expose this pig to further migration of ascarid larvae.

Unidentified:

We have seen a very severe eosinophilic hepatitis in our area, which we haven't been able to definitely attribute to ascarides. But it could be ascarides with a lot of fibrosis and this sort of thing.

Dr. Twiehaus:

We hope this paper coming out in the next AVMA Joural might answer this for us.

FOURTH SESSION: R. E. Omohundro,[1] Chairman

Paper No. 12

THE ROLE OF THE HOG CHOLERA VIRUS IN PRODUCING FETAL AND NEONATAL DEATHS IN SWINE[2]

By W. O. Cowart and L. G. Morehouse[3]

Sixteen sows were inoculated with attenuated hog cholera virus at intervals ranging from 30 to 109 days in gestation. A high incidence of stillborn pigs and mummified fetuses were observed in litters whose dams were inoculated before the 100th day in gestation. Ten sows in this category farrowed a total of 37 live pigs, 10 mummies, and 26 stillborn pigs. Six sows inoculated between gestation days 100 and 109 farrowed 57 live pigs, three mummies, and one stillborn pig. Five control sows farrowed 53 live pigs and two mummies.

Subcutaneous edema was the principal gross pathologic change noted in stillborn pigs. Sows inoculated between 70 and 109 days in their gestation period farrowed litters from which virus was demonstrated by fluorescent antibody (FA) techniques. Virus was demonstrated from spleen and tonsillar tissue of stillborn pigs and from pigs born alive that developed signs of hog cholera during the first 2 weeks of life. Splenic suspensions from these pigs inoculated into susceptible pigs rendered the latter immune to challenge with virulent hog cholera virus 20 days after exposure. There was no clinical evidence of spread of the virus from inoculated sows to contact susceptible pregnant gilts or their pigs.

Introduction

Attenuated hog cholera virus has been shown to produce malformations and other abnormalities in the fetuses of pregnant sows exposed before 30 days after mating (9, 24, 26). Gross lesions reported to occur in these fetuses were generalized subcutaneous edema, ascites, hydrothorax, hydropericardium, edema of the mesocolon and perirenal tissues, mottling of the liver, asymmetry of the head, lengthening and twisting of the snout, and malformations of the limbs. Pigs from sows given attenuated virus after the 25th day of gestation rarely showed malformations, but livability of these pigs was lower than normal. It was postulated that the placental membranes protected the pigs from infection with the virus after the membranes were completely formed at 28 to 30 days. Malformations were believed to be caused by the effects of the virus upon fetal organs during the time the organs were being formed (14).

Field observations indicate that hog cholera outbreaks may be initiated by infected pigs farrowed by sows exposed to attenuated strains or field strains of virus of low virulence during the gestation period. Susceptible, pregnant sows exposed to virus from the 20th to the 97th day

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[1] Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture, Hyattsville, Md.

[2] This report is taken in part from a paper presented before the regulatory section of the 104th Annual AVMA meeting in Dallas, Tex.

[3] Animal Health Division, Agricultural Research Service, USDA, Columbia, Mo., and School of Veterinary Medicine, University of Missouri, Columbia, respectively.

of gestation were reported to have farrowed pigs with cerebellar hypoplasia, hypomyelinegenesis, and congenital tremors (4). Hog cholera originating from prenatally infected pigs has been reported to have spread to older pigs on the same farm (3). Lesions occurring in baby pigs were subcutaneous edema, excess fluid in body cavities, "trembling," and hairlessness. Some pigs had characteristic gross microscopic lesions of hog cholera. Virus was demonstrated from affected pigs by fluorescent antibody tissue culture techniques (FATCT) and by inoculation of susceptible pigs. Other reports describe similar origins of field cases of hog cholera (5, 15).

This work was undertaken to observe, under controlled conditions, the effects of an attenuated hog cholera virus in susceptible pregnant swine at widely varying periods of gestation. Attention was given to clinical and gross and microscopic pathologic changes, as well as to detection of virus by FA techniques in fetuses, stillborn, and living pigs farrowed by exposed sows. Hog cholera antibody studies were conducted on the dams and piglets.

Materials and Methods

Experimental swine.--Eight-month-old gilts of mixed breeding were used for this study. They were not vaccinated for hog cholera and had not been in contact with vaccinated swine.

Source of virus.--Commercially available tissue culture modified live hog cholera virus (TCMLV) was used.

Quantitation of virus in tissue.--Virus concentration was determined by counting the number of fluorescent plaque-forming units (PFU) per milliliter of splenic suspension (12).

Cell cultures.--Pig kidney cell cultures (PK-15)[4] monolayers grown on coverslips in Leighton tubes were used for virus isolation and identification by FATCT (12).

Hog cholera antibody titers.--Antibody titers were determined by a method previously described (12, 15).

Frozen sections for FA tracing.--Frozen sections of tonsil tissue were stained with FA conjugate[4] using the Nebraska technique, a method previously described (1).

Experimental design.--Each of 16 pregnant gilts was inoculated intramuscularly (i.m.) with 4 cc. of attenuated hog cholera virus. The selected stages of gestation at inoculation were 30 days (2), 60 days (2), 70 to 82 days (3), 90 days (3), 101 days (3), and 107 to 109 days (3). One bred gilt each was placed in confinement with the groups exposed on gestation days 30, 60, and 90 and left there for 21 days to observe the possibility of spread of the virus from exposed to unexposed animals. Three bred gilts were maintained as unexposed controls. Pregnancies were allowed to proceed to term.

Results

Fetal mortality studies.--The 10 sows exposed before gestation day 100 farrowed a total of 37 live pigs, 10 mummies, and 26 stillborn pigs, for averages per litter of 3.7, 1.0, and 2.6, respectively. The six sows exposed after gestation day 100 farrowed 57 live pigs, three mummies, and one stillborn pig, for averages per litter of 9.5, 0.5, and 0.17, respectively (table 1). Five control sows farrowed 53 live pigs and two mummies--an average of 10.6 and 0.4, respectively. One control sow died of a condition not related to the experiment and was not pregnant.

Virus isolation and quantitation.--A pooled sample of splenic material from seven stillborn pigs in a litter whose dam was inoculated with virus at 70 days in gestation yielded a virus titer of 1610 PFU/ml. A virus titer of 27 PFU/ml. was demonstrated from one stillborn pig in a litter whose dam was inoculated at 101 days in gestation. Fourteen pigs from

[4]Obtained from E. A. Carbrey, Virology Diagnostic Services, National Animal Disease Laboratory, ARS, USDA, Ames, Iowa.

Table 1.--Fetal mortalities and virus identification in swine exposed to attenuated hog cholera virus at various periods of gestation

Number of sows exposed	Day of gestation when exposed	Total live pigs farrowed	Total stillborn pigs farrowed	Total Mummies farrowed	Pigs from which virus was isolated by FATCT			
					Pigs born alive		Stillborn pigs--	
					Number of pigs	Number of litters	Number of pigs	Number of litters
		Number	Number	Number				
2	30	4	[1] 2	0	0	--	0	---
2	60	1	2	9	0	--	0	---
3	70 to 82	14	7	0	1	1	7	1
3	90	18	15	1	2	1	0	--
3	101	27	1	2	3	1	1	1
3	107 to 109	30	0	1	8	2	0	--
Total--16		94	27	13	14	5	8	2

[1] One sow aborted on the 7th day postinoculation. One embryos was recovered.

five different litters developed clinical illness consisting of anorexia and lethargy. These pigs appeared normal at birth, but signs of illness were noted from 2 to 20 days of age. The dams of these pigs were inoculated at intervals ranging from 70 to 109 days in gestation. Virus was demonstrated from each of the 14 pigs. Virus titers ranged from 5 PFU/ml. to >10,000 PFU/ml. Virus was not demonstrated from piglets farrowed by sows inoculated with virus before the 70th day of gestation.

Gross pathology.--Subcutaneous edema, hydrothorax, and ascites were present in 60 percent of the stillborn pigs. The subcutaneous edema was 2 to 5 mm. in thickness. It was sometimes generalized, but was most frequently seen in the head and cervical areas. An occasional stillborn pig had areas of fatty degeneration in the liver, often involving an entire lobe. Three pigs had perirenal edema 2 to 4 mm. thick extending through the hilus into the pelvis of the kidney. Cerebellar hypoplasia was never seen.

Three pigs that appeared normal at birth, but sickened later, exhibited lesions associated with hog cholera such as petechiation of the kidney, epiglottis, and mucosal surface of the urinary bladder, erythema of the abdomen and inner surfaces of the legs, diffuse hemorrhage of the myocardium, and of the serosal and mucosal surfaces of the cecum and colon, peripheral accumulation of erythrocytes in lymph nodes, and edema of the mesenteric coils of the colon. Attempts to isolate bacterial pathogens from these animals yielded negative results. The course of the illness of these pigs ranged from 3 to 11 days at which time they were moribund and were killed.

Microscopic pathology.--Vasculitis was observed upon histopathological examination of the brains of the pigs that developed clinical illness. These pigs were farrowed by sows inoculated with attenuated hog cholera virus at 90, 101, and 109 days in the gestation period. Vascular lesions in these brains consisted of perivascular cuffing and endothelial proliferation which were characteristic of hog cholera infection. These changes were observed in the cerebrum, cerebellum, and medulla oblongata. Microscopic lesions attributable to infection with hog cholera virus were seen in the brains of stillborn pigs from one litter farrowed by a sow inoculated at the 70th day of gestation. There was endothelial proliferation and infiltration of mononuclear cells into vessel walls but frank perivascular cuffing was not observed. Brain lesions were not seen in stillborn pigs from other litters or from pigs killed at birth and shown to harbor virus. The latter were from sows inoculated subsequent to 90 days in gestation. This suggests that brain lesions may not be expected to occur regularly in stillborn pigs or in pigs not showing clinical signs of illness.

Serologic studies.--Antibody to hog cholera virus was not detected in serum samples from eight clinically ill piglets farrowed by sows inoculated with attenuated virus subsequent to 100 days. These pigs were from 13 to 20 days of age at the time of necropsy when serum samples were taken. All these pigs were shown to harbor virus. Antibody was shown to be present in serum from a clinically ill pig 8 days of age farrowed by a sow inoculated at the 90th day of gestation.

Virus transmission studies.--A pooled splenic suspension sample from seven stillborn pigs farrowed by a sow inoculated at the 70th day in gestation was injected i.m. into two 10-week-old susceptible pigs and one 4-week-old susceptible pig. These pigs did not sicken but were immune to virulent hog cholera virus when challenged 20 days' post exposure. Two 10-week-old pigs and two 4-week-old pigs were injected i.m. with splenic suspension from pigs from a sow inoculated with virus at 108 days of gestation. These pigs had become ill at 9 days of age. The 10-week-old pigs became listless and anoretic on the fifth day post-inoculation, but returned to normal by the 12th day. Both these pigs were immune to challenge after 20 days. One 4-week-old pig became incoordinated and anoretic and died on the seventh day post-inoculation. A virus titer of 31 PFU/ml. was demonstrated by FATCT from this pig. Viral antigen was demonstrated in frozen sections of tonsillar tissue by FA technique. The other 4-week-old pig died on the 18th day postinoculation. It exhibited no lesions but was thin and had failed to grow. Virus was not demonstrated from this pig.

Contact exposure.--Two sows, at 60 and 90 days in the gestation period, were exposed by contact to sows inoculated with attenuated virus at the same respective periods of gestation (table 2). The exposure period was 21 days, beginning on the day of inoculation. These two sows farrowed normal litters. Hog cholera antibody was not detected in sera of these sows or their pigs. One sow exposed to animals receiving virus on the 30th day of gestation died after 3 weeks of an unrelated condition. This sow was not pregnant when necropsy was performed. Hog cholera antibody was not detected in the serum from this animal.

Table 2.--Total numbers of pigs farrowed by sows with contact exposure only and by unexposed controls

Number of sows	Day of gestation on first day of contact exposure	Total live pigs	Total stillborn pigs farrowed	Total of Mummies farrowed
		Number	Number	Number
1	30	([1])	([1])	([1])
1	60	9	0	0
1	90	10	0	1
3	([2])	34	0	1

[1] Died, found not pregnant.
[2] Unexposed controls.

Discussion

This study supports the field observations of others that have pointed to transmission of the hog cholera virus from the pregnant sow to the fetus (3, 4, 5, 9, 15, 18). Earlier experimental work pointed to malformations and other abnormalities in the fetuses due to injection of modified hog cholera virus at the 14th to the 16th day of pregnancy (9, 14, 24, 25, 26). This appeared to fit a pattern for fetal abnormalities reported to occur in the human because of measles (Rubella) infection and in the ewe from exposure to modified blue tongue virus, in that abnormalities were attributed to infection during the first trimester of pregnancy (16, 20).

It has been postulated that in prenatal hog cholera infection the damage is done to the embryo before the placenta is developed to form a protective barrier (25). It has been pointed out that the developing swine embryo during the first 28 days of gestation would be vulnerable to infection since the placenta is not a complete entity until approximately 28 days after conception has occurred (7). Beyond this period, a much greater protection should be afforded the embryo since the five-layered swine placenta has been reported to serve as a barrier to passage of infectious agents or antibodies (10). This concept would suggest that exposure of susceptible sows later in pregnancy to either modified live hog cholera vaccine virus or to field strains of reduced virulence should not result in fetal abnormalities or high death loss. Two reports have described administration of modified live virus to immune sows late in pregnancy with no adverse affect on the litter (17, 23).

An increasing number of reports in recent years have pointed to various syndromes in neonatal pigs attributed to hog cholera field strains of reduced virulence or to attenuated virus in the dam (2, 3, 4, 5, 18). These suggest the virus is capable of crossing the placental membrane at widely varying periods of gestation. This study supports that belief. Although virus was not identified in pigs from sows exposed the 30- and 60-day intervals, a low livability and high incidence of stillbirths as compared to controls would indicate a virus involvement. Failure to detect it in offspring of these sows was possibly because of the small number of viable pigs available.

Lack of malformation in pigs involved in this study can probably be attributed to the fact that none of the sows were exposed before the 30 days in their gestation. Therefore, the virus would not have infected embryos at the most critical period of organ development. The marked subcutaneous edema and ascites occurring in nearly all stillborn pigs are unexplained. Sautter and coworkers (14) postulated the effect was caused by inhibition of the continued development and possibly the function of an organ or a system in the growing fetus. The role of the fetal-maternal relationship or the "placental barrier" in this type of infection is not fully understood. It is known that maternal antibody does not reach the fetus in utero in swine, whereas in primates there is apparently free passage of the intact Immumoglobulin G (IgG) molecule (8). It seems reasonable to assume that the hog cholera virus is not bound by the factors governing antibody transfer, since it in fact does gain access to the swine fetus. This passage of the virus to the fetus, however, must be subject to some selective mechanism. In this study, intrauterine infection was shown to occur in only 31.3 percent of the litters at risk of actual virus isolation. This supports the findings of other workers (3, 4, 5). It is also interesting to note that not all of the pigs in an infected litter were affected.

Both the FATCT and the Nebraska fluorescent antibody method appeared to be effective in identification of the hog cholera virus in this study. Other workers have indicated that modified live hog cholera virus may be detected by FA techniques (1, 11, 13). A method has been described recently for differentiating modified strains from virulent strains by FA tracing and employing the Nebraska technique (21). These workers reported that fluorescence became diffusely distributed throughout the lymphoid and epithelial tonsil tissue of pigs injected i.m. with virulent virus. Pigs injected i.m. with attenuated virus with or without 10 cc. of anti-hog cholera serum subcutaneously showed plaquelike areas of fluorescence in tonsillar crypt epithelium with infrequent individual reticuloendothelial cells in the diffuse lymphoid tissue and small plaques in the germinal centers that exhibited fluorescence. It was postulated that the type and pattern of fluorescence appearing in tonsil tissue depended upon the virulence of the virus, the dosage level, the susceptibility of the pigs, and other current stress factors.

In this study of pigs infected prenatally, plaquelike areas of fluorescence were seen in tonsillar crypt epithelium which often surrounded the crypt. In addition, plaques of fluorescing cells were seen in the surface epithelium and in germinal centers. However, there was never a diffuse fluorescence in all of the tonsil tissues as is observed in field outbreaks involving virulent strains. Variations in the pattern of fluorescence in this study from the previously described work might be expected since the route of infection was different, the dose received was unknown, and the fetus undoubtedly offered the ultimate in host susceptibility.

It is significant that hog cholera antibody was not detected in serum from pigs farrowed by sows inoculated after 100 days in gestation. This suggests that sufficient antibody did not reach the colostrum of the sows to be detected after its absorption by the pig. Neither did these pigs produce antibody, although virus was demonstrated from their tissues. This may have been due to a condition of immunologic tolerance. It is felt that antibody demonstrated from pigs harboring virus farrowed by a sow inoculated at 90 days in gestation was derived from colostrum.

Tissue culture modified live virus was used in this study as a model for gaining a greater understanding of prenatal hog cholera infection with specific attention to determination of persistence of virus in utero, means of detection, observations of fetal lesions, and clinical observations in surviving pigs. It should be emphasized that the findings reported here are applicable only to the agent used and the conditions prevailing at the time of this work and do not imply that the same results would be obtained with this or other agents under other conditions. Sautter and others (14) expressed their opinion on use of modified hog cholera virus as models for studying in utero infections by stating "it is important not to condemn these products unjustly without considering all aspects of their usage." The same would appear to hold true today. Concern has been expressed by others (3, 4, 5, 18) that persistent carrier or shedder pigs or both may develop from dams exposed to attenuated hog cholera virus during pregnancy. This possibility should now be minimized since the attenuated virus is not recommended for use in pregnant sows (22) Failure in this study to observe clinical evidence of spread of the virus to contact sows or their litters suggest losses would be confined to those specific litters whose dams had received the virus. This study, however, does indicate that the hog cholera virus is capable of passing the placental membrane at any period of gestation in susceptible sows, that it may be present in tissues of offspring in sufficient concentration to be detectable by the FA technique, and that clinical illness may develop in surviving newborn pigs. Such a situation could conceivably occur in naturally occurring exposure of susceptible pregnant swine to hog cholera virus of reduced virulence or through vaccination of animals of unknown status insofar as pregnancy is concerned. It would seem prudent to rule out these possibilities in undiagnosed cases of abortion in swine.

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DISCUSSION

Unidentified:

Was any serum used?

Dr. Cowart:

No. we didn't use serum, we used just virus alone.

Dr. Beveridge:

Did you test the virus you recovered from the pigs after birth to see if it had regained some of its virulence?

Dr. Cowart:

Only when we injected the virus into the susceptible pigs, which I mentioned, the 4-week old pigs and 10-week old pigs. Two pigs, that were 4 weeks old, died when we injected it into them. This virus was recovered from pigs that had shown clinical signs and typical lesions.

Dr. Twiehaus:

What was the incubation period?

Dr. Cowart:

In these pigs, one of the first ones died on the 8th day. He had been sick about 3 days.

Dr. Omohundro:

Dr. Morehouse, did you have something further to add?

Dr. Morehouse:

No, other than one of the reasons we have to stress the pigs used as a model is to try to determine what may happen to pigs in the field exposed to either field-type strains or possibly from misuse of hog cholera vaccine. The main reason here again for not using the serum is that we are trying to assess some of this antibody response.

EMBRYONIC DEATH, FETAL MUMMIFICATION, STILLBIRTH, AND NEONATAL DEATH IN PIGS OF GILTS VACCINATED WITH ATTENUATED LIVE-VIRUS HOG CHOLERA VACCINE[1]

By H. W. Dunne and C. D. Clark[2]

Summary

Vaccination of hog-cholera-susceptible gilts with attenuated live-virus vaccines and anti-hog-cholera serum at 24 to 60 days of gestation resulted in fewer pigs being born, more mummified fetuses and stillbirths, and fewer pigs surviving to 5 days after birth than were produced by non-immune and immune control gilts. Also, four of the six gilts pregnant for 24 days at the time of vaccination were barren at the time of hysterectomy. Increased embryonic death in the group of gilts vaccinated at 24 days of gestation was evidenced by the increased number of corpora lutea (CL) in excess of the combined number of live pigs and dead fetuses at hysterectomy, as well as the increased number of barren gilts. Virus was detected by fluorescent-antibody (FA)-cell-culture tests and by FA technique in frozen sections of pigs dying at birth or within 5 days after birth. Virus from infected pigs dying at birth was lethal when injected into colostrum-deprived 4-week-old pigs placed in contact with them. This confirms earlier theories and evidence that hog cholera virus can transverse the placental barrier to infect fetuses in utero. At birth, the newborn pigs infected in utero may retain the virus and become foci of infection in a susceptible herd.

Introduction

For years, small litters, mummified fetuses, stillbirths, weak pigs at birth and death of the pigs within 5 to 10 days after birth have been subjects of spirited discussions and pointed theories but the conditions remained inadequately explained. The late George Young (15) and coworkers (16), however, produced many of these conditions by using lapinized, live-virus vaccine against hog cholera in gilts pregnant less than 30 days. They demonstrated the presence of the virus in pigs surviving until term. Young expressed an opinion that the virus was not capable of passing the placental barrier after the 30th day of gestation. In Great Britain (9), however, gilts vaccinated with crystal violet vaccine and later exposed to virulent virus farrowed dead and dying pigs and weak pigs that died within a few days. Live hog cholera virus was demonstrated in pigs dying within 5 days after birth. It was reported (2) that pregnant gilts exposed to hog cholera could give birth to dead pigs, live pigs with congenital tremors or live pigs which later developed clinical hog cholera. Also, hog cholera could be transferred from sick, dying, and dead pigs to susceptible animals on the farm. In about 97 percent of the pigs with congenital tremors observed in Great Britain, the dams of the pigs had been infected with some agent immunizing them against

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hog cholera (8). In Minnesota, hog cholera transmission by pigs infected in utero occurred after the vaccination of gilts with modified live-virus vaccine during pregnancy (14). It was concluded that vaccine virus was the cause of the losses which occurred mainly in 2- to 4-week-old pigs (68 percent) and in some 9- to 10-week-old pigs (18 percent). Generally older pigs appeared resistant. Hog cholera developed in pigs inoculated with the blood from a sick pig and with tissues from an aborted fetus.

From 134 gilts of five herds in Indiana, (6, 7) 1,156 pigs were farrowed of which 455 (39 percent died of hog cholera designated as of prenatal source. Diagnoses were confirmed by the results of animal inoculation or fluorescent antibody (FA) tests. Congenital tremors attributable to the prenatal infection were observed in three of the five herds. Of 65 gilts (three were hog cholera immune) which were vaccinated while pregnant, only those vaccinated before the 20th day of gestation and those which were immune had normal litters (7). Mortality in all litters from gilts vaccinated between the 20th and 97th days of gestation ranged from 75 to 100 percent. A typical hog cholera was diagnosed in pigs on six farms where infection occurred in sows during pregnancy. Diagnosis was made using FA tissue culture and animal inoculation techniques (4).

In Germany, after inoculation of virulent hog cholera virus into hog-cholera-immune gilts a very weak virus was recovered from newborn pigs which suggested that the virus had been attenuated by intrauterine passage (10).

In Pennsylvania, hog cholera virus was detected by fluorescent antibody tests in a herd of pigs after the addition of two gilts which had been vaccinated with modified live-virus vaccine at about 75 days of gestation. These gilts were part of a group of six gilts vaccinated while pregnant, sold through a show sale, and distributed to four separate farms. Heavy mortality occurred on the four farms after the gilts farrowed. Several gilts maintained on the originating farm that were also vaccinated while pregnant, farrowed pigs which died and in which hog cholera was diagnosed by the FA test.[3]

The purpose of this paper is to verify experimentally that the live attenuated virus of hog cholera can penetrate through the placental barrier, infect pigs in utero, remain detectable, cause death in newborn pigs, and transmit lethal hog cholera infection by contacting hog cholera susceptible pigs.

Materials and Methods

First litter gilts used in the present experiments were primary specific pathogen-free (SPF) pigs obtained from a line of primary SPF gilts which were used as controls in other experiments. The gilts were raised in isolation and were moved to two outside breeding lots more than 4 weeks before breeding. Control gilts were kept in these lots after they were bred. The boar, also a primary SPF animal, was of the same linage, but not a littermate to the gilts used, and was kept in a small outside pen between, and adjoining the two breeding lots (to provide ample opportunity for all to develop a common bacterial and viral flora).

All gilts were "hand-bred" and "hand-fed" submaximal quantities of an approved ration once daily. Only those gilts that had not been observed to return to estrus were used. No exposure to vaccine virus was made before 23 days of the gestation period to permit the observation for estrus.

Bred gilts scheduled for exposure to live hog cholera virus by vaccination were moved to small outside isolation pens (with cement block and cement floor construction) located at least nine meters from any other pen and more than 30 meters from the breeding and control pens. To minimize fighting, the bred gilts were held in these pens with other gilts similarly exposed and from the same breeding lot. Group 1 comprising six gilts was vaccinated with attenuated live virus after 24 days of gestation. Group 2 consisting of five gilts was vaccinated after 60 days of gestation. Group 3 consisting of six gilts was the control group.

[3]Clark, C. D. Report of field cases of hog cholera associated with the vaccination of pregnant sows. Unpublished data. 1965.

Samples of blood were collected from the gilts before breeding, at the time of exposure to virus (vaccination), and at hysterectomy. The serums were examined for the detection of antibodies against hog cholera, leptospirosis, and brucellosis, and sometimes (if indicated) against other viruses such as pseudorabies (12) or SMEDI (Stillborn, Mummification, Embryonic, Death, and Infertility) (5) viruses.

Hysterectomies were performed on the gilts on the 112th or 113th day of gestation except two (gilts Nos. 93 and 21) which farrowed a day earlier. Pigs were removed from the uterus inside an operating hood and dried. Their navals were clamped and tied. The pigs were then placed in an air-filtered carrying box and taken to isolation units to be raised by using the SPF techniques (15).

Vaccinal virus used in the present experiments, with one exception, was a commercially produced, modified live virus of hog cholera attenuated in cultures of leukocytes and grown in procine kidney cells. The virus was administered in 2 ml. amounts and given with 7.5 ml. of commercially prepared hog cholera antiserum concentrate, according to that manufacturer's recommendations. The exception was the first four gilts exposed in group 1. These were given lapinized virus, two with antiserum and, unintentionally, two without antiserum. Originally, lapinized virus only was scheduled for vaccinating but additional vaccine was not available from the manufacturer.

Fluorescent antibody technique (1, 11, 13) was used for both antibody titration and virus detection. For antibody titration, serum was diluted twofold and incubated with 1×10^2 tissue culture doses of virus for 30 minutes before inoculation on to porcine kidney cell line (PK-15) grown in Leighton tubes. Cells on coverslips were allowed to react with fluorescein isothiocyanate conjugate after incubation for 24 hours. A negative reaction for antibodies was recorded when fluorescence in the test cells was as great as the fluorescence in the infected controls. Antibody titer endpoint was the point beyond which an adequate number of cells fluoresced because of infection by unneutralized test virus. Both the cell culture and the frozen section techniques (1, 11) were used to detect the virus in tissues of fetuses, stillbirth, and neonatal dead pigs. All uteri, fetuses, stillborn and neonatal dead pigs were examined by bacteriologic culture techniques at the time of hysterectomy or neonatal death.

Twenty-one gilts were available for the experiment; four were not used, however, because they returned to estrus after being bred a second time. (One of the four gilts, however, had eight healthy live pigs when slaughtered, see table 5.) In addition to those taken for the preparation of the serum for the FA tests, samples of blood were obtained from all of the virus exposed gilts (groups 1 and 2) at the time of vaccination and every day for 1 week and twice weekly for variable periods thereafter. Values for leukocytes, erythrocytes, hemoglobin, and blood cell volume were determined on both infected gilts and controls at the time of hysterectomy. Tissues from the uteri and spleens of virus exposed gilts and from all fetuses, stillborn, and neonatal dead were examined by FA test, both in frozen section and by cell culture.

Two 4-week-old colostrum-deprived pigs were inoculated with a 20 percent suspension of spleen from an infected pig. The source animal had died shortly after being born to gilt No. 19 which had been exposed to vaccine virus on the 60th day of gestation. Three pigs that had been given serum alone at 8 weeks of age were inoculated at 5 months of age with a virus containing suspension of tissue from a pig in an infected litter. Two colostrum-deprived pigs, 8 weeks old were inoculated with the same material. Three colostrum-deprived pigs 8 weeks of age were placed in contact with one of last-mentioned two pigs that had survived 60 days.

Data were tabulated on numbers of pigs that were born alive, were stillborn, and mummified, that died during hysterectomy, and that survived the first 24 hours, the first 5 days, and through 5 days. The 5-day period was an arbitrary selection but it minimized affects on the data because of occasional losses from other causes and losses associated with diarrhea at later dates. Survival rates from 24 hours to 5 days in control litters usually were excellent justifying the selection of the 5-day period.

The corpora lutea were counted in all gilts upon which hysterectomies were performed and which were not barren.

Results and Discussion

The effect of the vaccinal virus on pregnant gilts in the present experiment closely approached the findings in the field case reported by Emerson and Delez (6). Six nonimmunized gilts (group 1) were given attenuated hog cholera vaccine on the 24th day (table 1). Four of the six were not observed to return to estrus but were barren at hysterectomy. Another gilt (No. 44) had only two live pigs, two stillborn pigs, and one mummified fetus. Neither live pig lived as long as 5 days. The 6th gilt (No. 97), however, had an apparently normal litter with eight live pigs; she had no stillbirths and no mummified fetuses. Seven of the eight live pigs survived longer than 5 days.

The average litter size for group 1 gilts was 1.66 pigs. Antibody levels to hog cholera in the affected five gilts increased from negative in the serums of gilts before inoculation to as high as 1:512 in serum of the gilts at time of hysterectomy. The antibody titer of the unaffected gilt 97 remained negative throughout the experiment even though the gilt was exposed to the virus by vaccination and was kept in the pen and thus in contact with the other five gilts which had been vaccinated. Lapinized virus in gilts or pigs could not be detected satisfactorily by the FA technique either by the frozen section or cell culture systems.

In the group 2 (table 2) gilts that were exposed to vaccinal virus on the 60th day of pregnancy, the five gilts had 50 pigs (10.0 pigs per litter), 26 (52 percent) were mummified, and 5 (10 percent) were stillborn. Only 19 pigs (3.8 per litter) were born alive. The litter of gilt 24 is shown in figure 1. Only one of the 19 pigs born alive in the five litters survived as long as 5 days. One gilt was barren but the data could not be eliminated from the statistics because the gilt had not been observed to return to estrus after the first breeding and may have aborted unnoticed after exposure to the vaccine. (She was kept in a pen with other gilts which could have eaten the aborted fetuses.) The virus of hog cholera was detected in the tissues of stillborn and neonatal dead pigs (less than 5 days survival) by FA technique using frozen sections and infected cell cultures. Since only one pig in this group survived longer than 5 days in this group, attempts were not made to isolate the virus at periods longer than 5 days after birth. Tissue suspensions from one pig in the group that died soon after birth produced a fatal form of hog cholera when inoculated into susceptible SPF, colostrum-deprived pigs.

In contrast to groups 1 and 2, (exposed to vaccinal virus on the 24th and 60th days of pregnancy), the litters of group 3 gilts (table 3) comprised of 67 pigs (average 11.1 per litter) including three (0.5 per litter) mummified fetuses and one stillborn. Although 15 pigs died during the

Table 1.--Nonimmune pregnant gilts exposed to attenuated live-virus vaccines 24 days after breeding (group 1)

Identification of gilts		Composition of litter						Results of fluorescent antibody tests			
		Pigs born			Embryos over corpora lutea	Pigs surviving		Antibody titer in serum of gilts		Detection of virus in serums of pigs	
Gilt No.	Vaccinal virus[1]	Alive	Still-born	Mummi-fied		Pigs born over pigs alive at 24 hours	Pigs alive 24 hours over pigs alive at 5 days	Before vacci-nation	After vacci-nation	Tissue culture technique	Frozen tissue section
17	Lapinized vaccine.	Barren	--	--	--	--	--	Neg.	1:32	--	--
46	do		--	--	--	--	--	do	1:256	--	--
44	do	2	2	1	5/13	[2] 1/2 (1 IH)	0/2	do	1:64	Neg.	Neg.
14	do	Barren	--	--	--	--	--	do	1:64	--	--
32	Tissue culture vaccine.	do	--	--	--	--	--	do	1:512	--	--
97	do	8	0	0	8/9	[2] 8/8 (1 IH)	7/8	do	Neg.	Neg.	Neg.
Total		10	2	1	13/22	9/10	[3] 7/10	--	--	--	--
Ave. per gilt		1.66	0.66	0.16	6.5/11	1.5	1.16	Neg.	1:30	Neg.	Neg.

[1] 7 - 1/2 ml. anti-hog-cholera serum concentrated given to all but gilts Nos. 44 and 46.
[2] In hood (IH) -- died during hysterectomy procedures; lungs contained air.
[3] 70 percent.

Table 2.--Nonimmune pregnant gilts exposed to tissue culture-attenuated live-virus vaccine 60 days after breeding (group 2)

Gilt No.	Composition of litter						Results of fluorescent antibody test			
	Pigs born			Embryos over corpora lutea	Pigs surviving		Antibody titer in serums of gilts		Detection of virus in tissue of pigs	
	Alive	Still-born	Mummified		Pigs born over pigs alive at first 24 hours	Live pigs at first 24 hours over live pigs	Before vaccination	After vaccination	Tissue culture technique	Frozen tissue section
[1] 24	2	1	10	13/16	[2] 1/2 (1 IH)	0/1	Negative	1:256	Positive	Positive
20	5	2	5	12/13	[2] 3/5 (2 IH)	1/3	do	1:256	do	Do.
35	2	1	9	12/12	[2] 0/2 (2 IH)	0	do	1:128	do	Do.
19	10	1	2	13/14	[2] 4/10 (6 IH)	0/4	do	1:256	do	Do.
22	([3])			([4])	--	--	do	1:256	--	--
Total	19	5	26	50/55	8/19	[5] 1/8				
Average per gilt	3.8	1.0	5.2	12.5/13.8	1.6	0.2	Negative	1:230	Positive	Positive

[1] See figure 1. [2] (IH) Died in hood during hysterectomy procedures. [3] Barren. [4] No data (ovary in anestrus). [5] 12.5 percent.

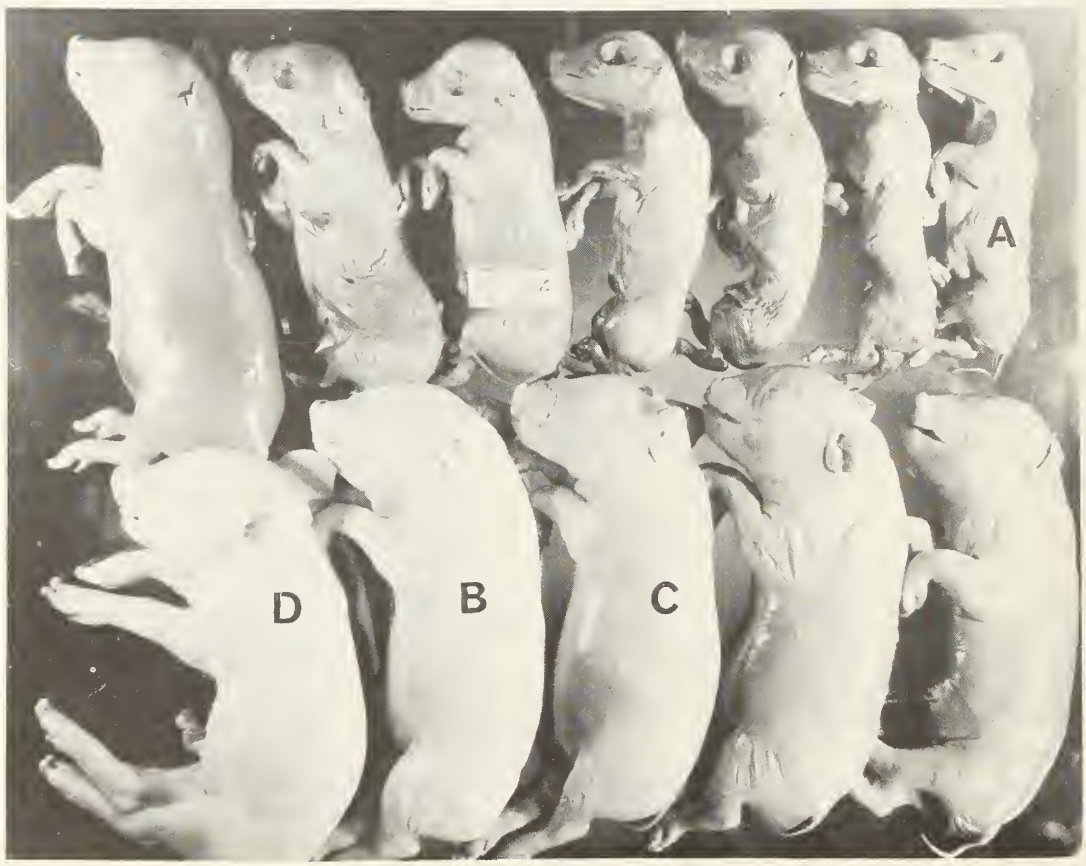

Figure 1.--With the exception of one live pig (not shown), the litter of gilt 24 that had been vaccinated against hog cholera with cell culture-attenuated, live-virus vaccine at 60 days of gestation (group 2). Notice that the youngest fetus dying was at a size comparative to a 65-day-old fetus (A). Notice also the gradation in size, the hemorrhages on the skin of one of the two larger pigs (B), that also had some "buffalo" characteristics, and the slight edema in the larger pigs (C). One of the two white pigs was born alive (D). The one with cutaneous hemorrhages was stillborn (B). All others had all been dead for 3 or more days.

Table 3.--Nonimmune pregnant gilts not exposed to live-virus vaccine (group 3)

Gilt No.	Composition of litter						Result of fluorescent antibody test			
	Pigs born			Embryos over coprora lutea	Pigs surviving		Antibody titer in serums of gilts		Detection of virus in tissue of pigs	
	Alive	Still-born	Mummi-fied		24 hours	24 hours through 5 days	Before pregnancy	After farrowing	Tissue culture technique	Frozen tissue section
[1]21	14	1	0	([2])	12	12/12	0	([3])	Neg.	Neg.
16	10	0	0	10/14	[4] 7(3 IH)	7/7	0	0	do.	do.
27	12	0	1	[5] 13/27	[4] [6] 6(6 IH)	6/6	0	0	do.	do.
231	10	0	0	10/13	[4] 9(1 IH)	7/9	0	0	do.	do.
98	8	0	0	8/10	[4] 6(2 IH)	4/6	0	0	do.	do.
45	9	0	2	11/11	[4] 8(1 IH)	8/8	0	0	do.	do.
Total	63	1	3	52/75	48	[7] 44/48	--	--	--	--
Average per gilt	10	0.17	0.5	9.75/12	8.0	7.3	0	0	Neg.	Neg.

[1] Farrowed naturally.
[2] No data.
[3] No sample.
[4] (IH)- died in the hood during hysterectomy procedures.
[5] 27 CL is an obvious case of superovulation and is not figured in average number of embryos over corpora lutea.
[6] Uterine artery ruptured prematurely, resulting in piglet hypoxis.
[7] 91.7 percent.

Table 4.--Survival percentage based on the number of ova released as shown by the number of corpora lutea formed

Group of gilts	Vaccinated	Surviving embryos at 30 days gestation	Surviving to parturition (born alive)	Embryonic survival to 5 days
		Percent	Percent	Percent
Group 1[1]	At 24 days	59.1	45.4	31.8
Group 2	At 60 days	90.9	29.1	1.8
Group 3[2]	Negative control	81.3	72.9	54.2

[1] 4 of 6 gilts were barren; data are limited to 2 gilts.
[2] Data do not include gilt 27 with 27 CL.

strip P-101

hysterectomy procedures, a total of 44 (65.7 percent of the 67 farrowed) survived 5 days. It was believed that pigs dying during hysterectomy, in both principles and controls more likely died of procedural inadequacies rather than acquired or genetic biological weaknesses though the latter were acknowledged as possible factors. Pigs of gilts in the control group that died had negative fluorescent-antibody-test results for hog cholera either in cell culture technique or in frozen tissue section.

After comparison of all groups (table 4), infection during early pregnancy, as in the group 1 gilts infected at 24 days of gestation, probably resulted in early embryonic death and absorption. In this group, of course, all survival rate is lowest, in part because of early embryonic death. Data in table 4 do not incorporate into the averages the negative effects of the four barren gilts in group 2, part of which, if not all, most likely were attributable to the virus.

The number of ova becoming fertilized and surviving to the time of parturition was seriously decreased by virus infection in group 1, 31.8 percent and group 2, 1.8 percent (table 4).

In the fluorescent antibody tests the spleen from infected pigs was a consistent source of virus detected by the cell culture technique. The results of the cell culture fluorescent antibody system were positive in all cases where the virus was detected by direct frozen section and in occasional instances where the virus was not detected by the latter system. Results of the frozen section technique were positive in 82.4 percent of the pigs which were shown to have virus according to the cell culture technique.

Several tissues were frozen and sectioned from each pig for FA tests. Not all tissues were FA test positive in each pig determined to have virus, but each tissue used had a positive reaction in more than one animal. In the order of decreasing rate of occurrence, positive test reactions by frozen tissue section were observed in the following tissues: pancreas, tonsil, kidney, liver, submaxillary salivary gland, lung, and brain stem. The uteri and spleens of gilts farrowing infected piglets were negative to both FA tests. The mandibular lymph node was positive on two of three times tried but nonspecific fluorescence in lymphatic tissue including the spleen made a valid positive diagnosis difficult.

The transmissability of the virus harbored in utero was proved by the infection of the two 4-week-old colostrum-deprived SPF pigs (pig Nos. 1 and 2, table 5) which were inoculated with the virus from an infected pig. The pig had died shortly after being born to gilt 19 which had been exposed to vaccine virus on the 60th day of gestation. Both inoculated pigs developed typical hog cholera symptoms and lesions and were positive to both frozen section and cell culture fluorescent antibody tests. The three 5-month-old miniature pigs (Nos. 3, 4, and 5, table 5) which had received passive immunity from serum alone 8 weeks before inoculation with the virus suspension, gave only a vaccination response and were subsequently shown to be immune. The two of five colostrum-deprived SPF pigs (Nos. 6 and 7, table 5), 8 weeks old, inoculated with tissues of the pig that died at birth developed temperatures of 41.4° and 41.6° C. One of the two inoculated pigs (No. 6) died 12 days after inoculation. The three pigs (Nos. 8, 9, and 10) that had been placed in contact with the infected pigs (Nos. 6 and 7) developed temperatures of 42.0° and 42.5°. Numbers 8 and 9 died at 29 and 30 days respectively after the others (Nos. 6 and 7) had been inoculated. The remaining two pigs--one inoculated (No. 7) and one contact (No. 10)--continued to have

Table 5.--Inoculation of hog cholera susceptible pigs with virus from newborn pigs infected in utero

Pig No.	Route of infection	Course of disease	Termination	Fluoresent-antibody test for hog cholera
Group A:[1]				
1	Subcut. inoc.	16 days	Death	Positive.
2	do.	24 days	do.	Do.
Group B:[2]				
3	do.	No illness	Recovery- immune	Not done.
4	do.	do	do	Do.
5	do.	do	do	Do.
Group C:[3]				
6	do.	12 days	Death	Positive.
7	do.	63 days	do	Do.
8	Contact	29 days	do	Do.
9	do.	30 days	do	Do.
10	do.	75 days	do	Do.
Group D:[4]				
11	Contact with pig nos. 7 and 10 60 days after exposure to virus	21 days	do	Do.
12	do.	23 days	do	Do.

[1] 4-week-old colostrum-deprived SPF pigs.
[2] 5-month-old pigs that had been given anti-hog-cholera serum 8 weeks before inoculation.
[3] 8-week-old colostrum-deprived SPF pigs.
[4] 14-week-old colostrum-deprived SPF pigs.

sporadic temperature rises. At 63 days postinoculation, 3 days after two additional pigs (Nos. 11 and 12) of the same age were put into the pen, the original inoculated pig (No. 7) died and had lesions of hog cholera. The virus was detected in its tissues. The last of the original contact pigs (No. 10) died of hog cholera at 75-days-post contact. The two additional pigs (Nos. 11 and 12) developed high temperatures reaching a peak of 42.0° C. in 4 days. They died 21 and 23 days after contact.

Results of examinations of bacterial cultures made of all fetuses stillborn or dying pigs during hysterectomy procedures were negative. Bacteria isolated from the intestines of pigs living from 1 to 5 days included E. coli, Klebsiella sp., Seratia sp., and Pseudomonas sp. None were uniformly present. Results of serologic tests for leptospirosis and brucellosis in the gilts were negative.

Histopathologically, perivascular cuffing of blood vessels occurred occasionally in the heart, brain, and liver of virus infected newborn pigs. Malformations were not observed in these pigs, most of which were from gilts infected with virus at 60th day of gestation. One gilt (No. 24, group 2) had marked hemorrhages of lymph nodes and marked infraction of the spleen. Virus was not detected in the spleen of the gilt by FA test.

Corpora lutea counts have been found in other unreported experiments to be useful in approximating early embryonic deaths. In eight farrowing gilts in the present experiment, including both gilts infected at 60 days of gestation and controls (groups 2 and 3) and on which data were available (with one exception explained below), there were 14 more CL than there were live, stillborn, or mummified pigs. Thus, there was the average of 1.7 CL (with a range of 0 to 3 CL) more than the number of pigs per litter. In contrast, group 1 which were given virus 24 days after breeding had only two gilts which farrowed a total of 13 pigs and had 22 CL. One gilt had a total of five living, stillborn, and mummified pigs, and 13 CL. The other (gilt No. 97), previously described as having a normal litter, had eight pigs and nine CL. The average was 4.5 more CL than pigs per litter. The exception cited above was a negative control gilt (No. 27) which, apparently through super ovulation, had 27 CL. The largest number of CL previously observed in a first litter sow in this laboratory was 19.

Barreness in four of 6 gilts of group 1 not observed to return to estrus before inoculation with viruses on the 24 day of gestation also was specially studied. Comparison of breeding records of all gilts bred twice and all found to be barren is shown in table 6. Four of 21 gilts were not used in the experiment because they had been bred twice and returned to estrus. At least three had normal estrus cycles. Two of these three had incompletely form oviducts. The third appeared normal. All three were barren. The fourth gilt had eight normal fetuses when slaughtered at about 65 days of gestation. Apparently it had been observed in false estrus or mistaken to be in estrus a second time. The fourth gilt had 13 CL which may have been caused by an abnormal ovulation during pregnancy, hence a false estrus.

Table 6.--All gilts returning to estrus or subsequently found to be barren

Gilt No.	Number of times bred	Return to estrus after second breeding[1]	Days return to estrus after first breeding[1]	Experimental use	Litter size
30	2	Yes	17	Not used	Barren
18	2	Yes	20	do	Do.
36	2	Yes	17	do	Do.
26	2	Yes	([2])	do	8[3]
27	2	N.o.	25	Control	12
131	2	N.o.	26	do	10
17	1	--	N.o.	Group 1	Barren
46	1	--	N.o.	do	Do.
14	2	N.o.	([2])	do	Do.
32	2	N.o.	18	do	Do.
22	1	--	N.o.	Group 2	Do.

[1] N.o. none observed.
[2] First breeding date not available.
[3] Slaughtered on the 65 day of pregnancy, thought to be barren.

Irregular estrus cycle in gilts after breeding and barreness with no observable return to estrus seemed to be typical signs of hog cholera virus infection during the first 24 days of pregnancy. It also seems that a marked difference between the number of CL and the combined total of live and mummified fetuses would reflect that a virus infection exists. These signs also were observed with the "SMEDI" enterovirus (5) infections of the gilt, and somewhat similarly with pseudorabies infections. Mummification of fetuses, stillbirth, and early neonatal death frequently occurred in sows infected with the virus in the early pregnancy period but was observed most commonly in hog cholera when infection was initiated later than the 30th day of gestation. Time of exposure to virus could be approximated in unknown cases by the size of the smallest mummified fetus. Since the bony skeleton did not decrease in size, it was compared to formalized specimens of normal fetuses removed at known times. Assuming comparative intrauterine development and assuming the need of not less than 4 days, virus infection to produce fetal death, it was possible to determine, within a range of a few days, the latest (but not the earliest) time that infection could have taken place. The presence of more than three excess CL per litter, however, may indicate that viral infection occurred before the 28th day of gestation. Twenty-eight days is approximately the latest time at which the skeleton remains sufficiently uncalcified and can be completely absorbed at death. The smallest mummified fetus observed in our laboratory was approximately 1.25 cm. long and was identifiable only by head and rib marks. These generally were designated 28-day-old mummified fetuses. Development before 28 days was considered embryonic because complete absorption was possible.

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DISCUSSION

Dr. Whitehair:

I would like to ask what was your third to last conclusion when you said something about immunization to this virus?

Dr. Dunne:

I believe you are referring to the 5-month old pigs that had received serum at 8 weeks, 8 weeks before their exposure. They had 8 weeks to lose their passive antibody. We figured they ought to be susceptible at this point. They showed a slight reaction to the FA test. They were immune.

Dr. Gustafson:

From a little conversation at the table, it was indicated that Bufficine[1] was being used. I would like to point out that this is not a leukocyte vaccine. The virus was attenuated on leukocytes, but the company producing this particular vaccine used primary kidney cells. I think this is an excellent indication of what kind of trouble one can get into by using primary cells for the development of vaccine, or for the commercial production of it. It certainly was attenuated on leukocytes, but then grown on primary porcine cells. This way they got into trouble with it.

As a matter of fact, I have done some work with the virus that I have in the laboratory in pregnant sows. I wish you would try that. This is a matter of my personal opinion, because of our success in passing on to embryos in five sows. We have only done five. Consequently, I didn't feel it was worthwhile to report on that small number. In 1965, we did two sows. One raised 13

[1] Eli Lilly and Company; hog cholera vaccine, modified live virus, tissue culture origin.

of 16 pigs and the other, 10 of 12 pigs to weaning. All these pigs were susceptible at the end of that period. In the pigs that were dead, we made efforts to demonstrate hog cholera virus in each by removing the spleens within an hour or two after they were born. We took the spleens out promptly and put them into susceptible pigs that day. Those pigs ultimately died when they were challenged with virulent virus. So we were unable to demonstrate virus in those and the pigs were susceptible. We did three others with similar results. Of these, one raised five of eight and 12 of 16 in another. The third sow raised six of 13, but she lay on three of them. We were unable to demonstrate virus in any of these embryos. We tried to get to them as quick as possible to see if we could find whether or not this virus was present in the embryo. We were unable to demonstrate on that basis. But this was a matter of personal interest. I thought it would be nice if I had an opportunity to point out that the Bufficine vaccine is somewhat different than that provided to companies to start with.

Dr. Dunne:

Just as a matter of curiosity, at what ages did you vaccinate the pigs?

Dr. Gustafson:

This was done at approximately 21 days after breeding. We waited until they were past their heat period and then we exposed the sows at that point. They were bred; then we waited to see if they passed their next heat period.

Unidentified:

I have the impression from the two previous speakers that it doesn't make any difference if you use lapinized vaccine or the tissue culture vaccine according to what you get out of the baby pigs. Is this correct? I would like to ask you, Dr. Morehouse?

Dr. Morehouse:

It doesn't make much difference what the source of the virus is. If the virus is still virulent, it will cause some damage. I don't think we have all the answers now based only on our experience with five sows, as this is a very limited number.

Dr. Dunne:

I agree with this, at least to a degree. I think it will show, when we get to the SMEDI viruses, that pathogenicity to the sow may not necessarily be the entire picture. So we may not be able to extrapalate this type of hog cholera. There may be a difference in the two viruses. When we get to the SMEDI viruses, I would like to point out, that here we are using a virus that has absolutely no effect upon the sow, or appears to have no effect at least, and yet there is a very definite effect upon the fetus. So, pathogenicity to the sow may not be the same as the pathogenicity to the fetus.

Dr. Carbrey:

I was given to understand, from my biologic contacts, that they put your virus into a pig, and had a pig passage, then made a tissue culture from this kidney. Is that right?

Dr. Morehouse:

I presume they put it into a pig to infect the kidney, then they grew the kidney cells out. I think they got in trouble this way. The product they have now is grown on leukocyte cell cultures that I provided. It is one of those vaccines that did pass the test to be used currently. Dr. Phillips gave a good report on this at the USLSA meeting this past year.

Dr. Tillery:

The product that Dr. Clark used is not the product presently being marketed. That product was discontinued several months ago.

Dr. Torrey:

This has quite a lot of interest to me especially, I think because in the early years of experimental work on hog cholera this is one of the questions that was very important at that time. About 1916, Dr. Niles ran experiments with the old simultaneous method to determine if they could vaccinate sows. As far as I know the results were never published, but it is in some of the old records. They vaccinated over 200 head of sows with serum and virus, and the records show that there was very little detrimental effect on vaccinated pregnant sows. There was no record to show at what age they were vaccinated, but I would assume that all different ages would have been involved because that would not have been an important factor to them. They went on record as recommending this as a standard procedure. I think those of use who have been in this for some time remember that very little attention was paid to vaccinating pregnant sows, I don't think the records show that there is any great detrimental effect. The question I would like to raise is: Isn't this probably a difference in pathogenicity? I think there are some other factors involved. I realize there was serum used with this virus and this may be an important factor in using modified vaccine. So I think the modified vaccine is very different from virulent virus. These viruses have been changed so markedly in the production of them, and in changing from one highly pathogenic to all different stages of pathogenicity so there is a difference in the virus. I think attenuated virus is much more tenacious than the lifespan and conditions under which it survives and all these things compared to virulent virus. I realize too, that virulent virus was either all or nothing. We didn't have all these things in between in the animal. There wasn't an opportunity to observe what might have been the condition. But, if we accept the work of some recent publications from Germany in which they have tested virulent viruses at different dates and times in the development of the animal, they have shown that in the latter stages of the disease--assuming that the animal dies, or if it is a kind of chronic case--they have been able to demonstrate that these viruses lost their pathogenicity in the animal that might have died. These are some of the things that are involved in studies of this type of virus.

Dr. Dunne:

Dr. Torrey, I couldn't agree with you more on your reactions to the virulent virus. I have observed virulent virus and agree to its causing any problems with the fetus. I do want to comment on the work that you were citing about the attenuation of the virus by its staying in the pig for a long time. This is Korn's work and I don't agree with Korn that this happens as a constant thing. For about 2 years I tried to attenuate hog cholera virus by picking the virus at the later stages of chronically ill individuals. I think, periodically, it comes back into an acute stage. It would go along in one animal and would run out to about 30 days before the animal died. I would take this virus and the next animal would die at 10 to 12 days. You might get a couple of them that would go

chronic. I would think we have an attenuated virus, then it would go back again. Perhaps if we did this for a number of years it might happen. I don't really believe that we can categorically state that this happens with the virus. I think again that what happens is not the attenuation of the virus; many things are happening with the virus and I don't think this is a constant feature. It may happen, but it certainly isn't constant.

Dr. Gustafson:

I'd like to add a comment to that story. From our experience and others, if you have a pig that is chronically infected and move him, that is, change from an outside house to an inside room, or from an inside room to a cold place outside, that pig will die in just a few days--apparently from the stress of environment. A nice way to establish a chronically infected pig is to dilute virus (virulent virus, that will kill pigs in 10 days) out to 10 point and have a 10 to the minus six or 10 to the minus seven virus infection in that pig. That is a nice way to start a chronically infected pig, because they seem to live longer the farther out one dilutes the virus.

Dr. Dunne:

This doesn't happen always either. This is the way to start it. You have to get the right one. I think that what happens is that the virus is a conglomerate of different particles. If you happen to get the right particle, this will happen. But I diluted it out in one instance. The highest dilution we have ever gotten from a hog cholera virus is 10 to the minus 9, and this only once. One pig out of three did that. I did the dilution myself. We were particularly interested in this one. One out of there pigs came down with it with 10 to the minus 8; all the pigs in this titration died. At 10 to the minus 9, one died. The catch in this, of course, there must be pathogens. We were diluting old blood and obviously we got one blood cell, a red blood cell in this 10 minus 9, and one pig died. He died with lesions of hog cholera. Now we have also diluted it down and had chronic pigs develop. I think it is a matter of getting down to an individual virus particle or infective group of particles, which are not so virulent as when we take the field viruses in certain mixed populations of virulence.

Dr. Gustafson:

Dr. Beveridge, how long has it been since they stopped all vaccination in England? I have forgotten just when it was. It has been a year or more, hasn't it?

Dr. Beveridge:

I think that vaccine has been stopped for maybe 3 years now.

Dr. Gustafson:

I thought I remembered. I was there in May of this year, and talked with Dr. Done. When I returned, I found that Britain has been declared hog cholera free. They no longer have hog cholera. Well, there are two things I remember from this conversation. One was that they insisted that they stop all vaccinations. As a result of this there should not be antibodies in the animals. It would be much easier for them to trace down outbreaks, destroy the animals, and get rid of the infection. But then they found that the real resistance came from places where the animals would

be brought together for resale. They anticipated this, and it did occur. They destroyed those animals and now these people are very much interested in having the people from Weybridge being involved in their activities.

Dr. Done says they still continue to find occasional animals with antibodies, although they have not used vaccines for over 2 years. They wonder now where these antibodies are coming from since vaccines are no longer used, and they are hog cholera free. So, while they are hog cholera free, they still have a few animals around that are developing antibodies.

Dr. Dunne:

Well, I rather suspect a very similar situation will occur in this country if we ever get to the point where we eliminate all vaccination. There will be a few "diehards" who will stock up with vaccine. England is not going to be in our situation because they did not have the lay vaccination that we have here. We must assume, also, that there are veterinarians, and there will be a few of them, who will not wish to give up vaccinations completely, but will want to wait and see what others are doing. There will be a certain amount of residual vaccinations going on.

Let me make just one other comment here regarding the Chinese strain. I talked to the veterinary group in Paris last May. At this time I was confronted with the same question, why didn't we use the Chinese strain if it was so much better? I agreed that the reports were wonderful. I asked at that time if anyone had tried it in pregnant sows. I didn't get any positive answer at that time.

Dr. Twiehaus:

I would like to call your attention to work done by Dr. Potter in Minnesota in 1953, where they used immunized sows, or different vaccines, and then introduced the modified vaccine on top of the immunized sows, and had abnormalities. So, whether the animals are immunized or not doesn't always preclude the fact that we still don't get some abnormalities.

Dr. Dunne:

I must comment on his because I think that there could be some irregularities associated with that type of experiment. First of all, vaccination doesn't preclude immunization. Since they didn't really have means in those days, except challenge of knowing whether the animals were immune, I doubt that these animals we worked with were challenged before subsequent exposure.

In the preliminary work we are doing with the SMEDI and hog cholera viruses, we have yet to find the sow that responds to a second exposure to the virus after she has been immunized and satisfactorily develops antibody. We have also checked this out on animals that were brought in through a given herd, where the animals were immunized before they were brought in. Here we are taking the same chance that we took in the experiment you cited, in that we didn't check the animals for immunity. But they were vaccinated with attenuated vaccine before they were brought in and were also revaccinated. There were no problems. I don't believe that this observation is a completely valid one at this point. I think if we find immunity and if you have a real immunity in the individual, that the individual will not subsequently be infected with the virus. If you look at it this way, as long as you have antibodies present in the system you can't establish a viremia. This is what happens with serum block. As long as there is an adequate number of antibodies in the system you can't establish a viremia. If you can't establish a viremia you are not going to get any response.

Dr. Carbrey:

Dr. Stewart has some work with immune sows, which I think may clear up this point when he presents his paper.

I was fortunate to visit England for about 9 weeks in fall of 1964. They had quit vaccinating with crystal violet in summer of 1964, so it must be close to 3 years now. England had about six herds in which they thought crystal violet vaccine had masked the pregnant sow transmission, but actually only proved it in one, I think. So, at first it was quite a scare to them. On the other hand, they raise about 13 million pigs a year. At the peak of their crystal violet immunization, they only got about one-half million vaccinated. So they thought it wasn't really doing anything for their program. I attended a meeting at Liverpool, and practitioners there were quite upset to see crystal violet vaccine discontinued.

Dr. Ray:

I will say this, sows that you expose to these vaccines vary. One veterinarian will be vaccinating sows. One time he vaccinates a group of sows that are pregnant, nothing happens. The next time he vaccinates them, trouble breaks loose. I think that is one of the things Dr. Young found in his work. Now, we have never recommended our vaccine in pregnant sows, but we know it has been used a few times. I remember one time even before we had a license to produce it, where a veterinarian vaccinated 100 sows, they never had a problem. Another vaccinated 60 and never had a problem. I have tried to get information from fellows who have used it on pregnant sows. We have never had any trouble reported, but they are not using it so they shouldn't have had any trouble. One bunch of sows will react one way and another another way, so Gustafson may have had some good sows to work with. I know that our vaccine will transmit if you keep them in small enough pens. That has something to do with it. Apparently if you get the right kind of sow it will transmit. Also, I think Dr. Young, in his work in Indiana, has found definitely that some of these sows might not have been immune. However, they were vaccinated or exposed to vaccinated pigs and have come up with litters that had persistent infection, infected with modified live virus vaccine. So, in the field, I believe they have considered exposed pregnant sows to be a potential problem even though these sows have been vaccinated.

THE INCIDENCE AND CHARACTERISTICS OF STRAINS OF HOG CHOLERA VIRUS CAUSING FETAL ABNORMALITIES, DEATH, AND ABORTION IN SWINE[1]

By E. A. Carbrey, W. C. Stewart, J. I. Kresse, and L. R. Lee[2]

Summary

Laboratory findings were compared with clinical histories on 64 herds with baby pig losses, abortions, and fetal abnormalities. Hog cholera (HC) virus was isolated from 18 of these herds. When the field diagnosis was uncomplicated myoclonia congenita, HC virus was isolated from only one of 17 herds.

Hog cholera virus strains from 11 of these herds were characterized by inoculation into susceptible pigs. Five strains were avirulent, three were of low virulence, and three of high virulence. One of the low virulent strains produced a chronic course of disease in the pig lasting 93 days. The pig had periods of normal clinical appearance during this time, although virulent HC virus could be recovered from its blood, and contact swine consistently succumbed to fatal infection with HC.

The role of hog cholera virus in producing the undesirable clinical signs listed in the title of this symposium has been established (2, 3, 4, 6). However, the relative importance of intrauterine HC infection of baby pigs as compared with genetic, nutritional, toxic, bacteriologic, and other factors has been a source of concern to the regulatory veterinarians engaged in the eradication of HC. The HC diagnosticians have been encouraged to submit specimens to the laboratory from swine herds with disease problems of this nature. Although the primary concern of the laboratory was the isolation of HC virus, a sufficient number of cases have been processed so that some interesting information has been obtained. A summary of these accessions is presented.

Some characteristics of strains of HC virus involved in baby pig losses are not the same as those of strains that cause an acute and fatal infection in susceptible pigs. Obviously, for a HC virus strain to cause the syndrome under study the affected sow must survive the initial infection. The virulence of the virus must be that of a sublethal or avirulent strain.

The persistent virus infection of the prenatal pig is quite similar to the immunologic tolerant state observed in mice infected with lymphocytic choriomeningitis (LCM) virus. However, only certain strains of LCM virus consistently produce this unusual type of virus infection (5). A number of HC virus strains isolated from swine herds with baby pig losses were inoculated into susceptible pigs, and the severity of the disease was observed. One of the virus strains produced a syndrome resembling immunologic tolerance, and most of the strains were found to be of reduced virulence. These findings are presented in some detail as evidence that the HC virus strains causing the clinical signs of interest to this symposium have different characteristics, particularly with respect to virulence.

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[1]The authors acknowledge the assistance of the Swine Diseases Staff and field veterinarians of the Animal Health Division, ARS, USDA.

[2]All members of Animal Health Division, Agricultural Research Service, USDA, National Animal Disease Laboratory, Ames, Iowa.

Materials and Methods

Specimens

An effort was made to obtain tissue specimens for virus isolation from as many swine herds as possible with a history of fetal malformations, abortions, and baby pig losses, even though HC was not the disease primarily suspected. Herds with a history of stillborn pigs or deaths in baby pigs under 3 days of age were included. A history of "trembling, shaking, or dancing" pigs was of particular interest to the field diagnosticians.

Tissues, usually spleen, tonsil, and lymph node, were prepared for inoculation on coverslip PK-15 (pig kidney) cell cultures as clarified, 33 1/3 percent, suspensions in Earle's medium plus 0.5 percent lactalbumin hydrolysate and antibiotics. A part of the suspension was frozen and saved for pig inoculation. The cultures were examined for HC virus by the fluorescent antibody, tissue culture technique (FATCT) at 24 and 48 hours following inoculation (7). Many of the specimen tissue suspensions were cultured for other swine viruses on primary swine kidney cell cultures through three blind passages. Additional differential examinations for bacteria and toxic agents were performed on some of the specimens. The concentration of HC virus in the suspensions was determined by a fluorescent plaque counting technique previously described (1).

Pig Inoculation

First or second generation specific-pathogen-free (SPF) HC susceptible pigs weighing approximately 40 pounds (18 kg.) were inoculated intramuscularly (i.m.) with tissue suspension and maintained in isolation until death. If the pig survived, it was given 1.0 ml. of virulent HC virus suspension i.m. containing at least 10,000 plaques/ml. by FATCT. Usually only one pig was inoculated, and the virus strain was classified as follows:

High virulence--Pig sickened and died. Lesions of HC were observed on necropsy, and HC virus was recovered from the tissues by the FATCT.

Low virulence--Pig had chronic illness and recovered, or died after a protracted course in which periods of remission occurred. Lesions of HC were not severe, but HC virus was recovered from the tissues.

Avirulent--Pig had little or no reaction following inoculation and remained healthy following inoculation with virulent HC virus.

Results

A total of 64 submissions were received from herds with histories of baby pig losses from January 1, 1966, through August 30, 1967. Most of the specimens were from baby pigs, but some of the tissues received were from older pigs. The herds were tabulated according to laboratory findings and clinical histories (table 1).

The clinical signs were classified according to the three headings in table 1 as follows:

Malformations and nervous signs--Under this category were included all fetal abnormalities, both premature births and fullterm, and the snydrome of myoclonia congenita, "dancing," "trembling," and "shaker" pigs.

Abortions--Premature expulsion of pigs from the uterus.

Deaths in first 3 days of life--In this group were included stillborn pigs coming fullterm as well as those dying shortly after birth. Many herds with histories of baby pig losses were excluded from the survey because the affected pigs were more than 3 days old.

Table 1.--Laboratory findings compared with clinical histories on 64 herds with baby pig losses

Laboratory findings	Cases	Clinical history		
		Malformations and nervous signs	Abortions	Deaths in first 3 days of life
	Number	Number	Number	Number
Hog cholera virus	18	6	9	15
Transmissible gastroenteritis agent	1	0	0	1
Hemolytic streptococcus	1	0	1	1
Chronic arsenic poisoning	1	1	0	0
Nitrate poisoning	1	0	1	0
Negative	42	22	14	35
Total	64	29	25	52

Table 2.--Field diagnosis compared with laboratory findings on 58 herds with histories of baby pig losses

Field diagnosis	Cases	Laboratory findings		
		Negative	HC virus	Miscellaneous
	Number	Number	Number	Number
Myoclonia congenita	17	15	1	1
Hog cholera	30	14	14	2
Unknown	11	11	0	0

Hog cholera virus was isolated from 18 of the 64 herds, or better than a fourth. Death in the first 3 days of life was reported from nearly all HC virus positive herds, 15 out of 18. Nine of the HC infected herds had abortions, but only six reported malformations and nervous signs.

The agent of transmissible gastroenteritis was detected by pig inoculation in specimens from a herd having only deaths in baby pigs. However, the affected pigs were less than 3 days old. The herd with the laboratory findings of chronic arsenic poisoning had "trembling" pigs without the usual deaths in the affected pigs. Abortions were the only clinical signs reported from a herd in which nitrate poisoning was determined by toxicologic examination. A majority of herds, 42, had negative laboratory findings, although the histories indicated severe baby pig losses.

The diagnosis made in the field or the disease condition suspected by the veterianarian provided an interesting comparison with the laboratory findings (table 2). Where HC was suspected, the laboratory confirmed with virus isolations in half of the cases, 14 out of 30. However, when Myoclonia congenita was diagnosed with no reason to suspect HC, the laboratory findings were negative on 15 out of 17 submissions. Hog cholera virus was isolated only once from herds where uncomplicated Myoclonia congenita was reported.

Pig inoculations were performed with tissue suspensions from specimens received from 11 of the herds from which baby pig losses were reported. The findings are presented in table 3 in the order of age of pig from which the tissues were collected, from aborted fetuses to 8 weeks of age.

Of the six HC virus strains obtained from aborted fetuses and pigs less than a week old, two strains were of low virulence and the rest were avirulent or immunizing strains.

One of the strains isolated from aborted fetuses, 17637, produced a chronic infection in the pig which lasted 93 days (table 4). This pig, which weighed 24 pounds and was 10 weeks

Table 3.--Characteristics of HC virus strains isolated from herds with abortions, fetal abnormalties, and baby pig deaths

Herd No.	Age of specimen pig	Spleen plaque counts	Pig inoculation		Source of virus
			Death, days post inoculation	Virulence	
17637	Aborted fetuses	>10,000	93	Low, (immune tolerance)	Unknown
27922	do	>10,000	Survived	Avirulent	Do.
20542	1 day	[1] >10,000	do	do	Do.
16112	1 day	>10,000	do	do	Vaccine (live)
17670	1 day	>10,000	24	Low	Unknown.
27239	5 days	>10,000	Survived	Avirulent	Vaccine (live)
27547	2 weeks	2,700 9,000	15	High	Unknown.
19796	4 weeks	>10,000	Survived	Avirulent	Vaccine (live)
16064	5 weeks	Positive	29	Low	Do.
18892	7 weeks	>10,000	8, 20, 23	High	Do.
17010	8 weeks	43 & 90	18	do	Do.

[1] Lung instead of spleen.

Table 4.--Clinical course of hog cholera infection in a pig resembling immunologic tolerance, herd 17637

Days post inoculation	Clinical signs	Total white blood cells per cmm.	Hog cholera virus isolations
9	Anorexia	--	Feces - positive
14	Distress, anorexia, clonic spasms	--	Blood - >10,000/ml.
19	Normal	3,550	Blood - >10,000/ml.
30	Slight anorexia	5,700	Blood - >10,000/ml.
33	Normal, good appetite	--	--
37	Normal	4,550	Blood - 12,000/ml
44	do	6,880	Blood - >10,000/ml.
51	do	--	Blood - >10,000/ml.[1]
54	Fever, anorexia, weakness	9,750	--
58	Posterior paresis	6,950	Blood - >10,000/ml.
61	Improved, but weak	--	--
82	Lacrimal discharge, normal otherwise	--	Blood - >10,000/ml.[1]
92	Anorexia, diarrhea, posterior paresis	--	--
93	Dead	--	Spleen - >10,000/ml. Tonsil - >10,000/ml. Lymph Node - >10,000/ml. Heart Blood - >10,000/ml.

[1] 100 percent of cells on coverslip culture contained HC virus.

of age, was given 5.0 ml. of the composite splenic suspension subcutaneously. At 9 days post-inoculation (DPI), the pig had anorexia and a fecal specimen was collected. HC virus was isolated from this specimen by the FATCT. The pig was off feed, appeared distressed, and had clonic spasms on 14 DPI. By 19 DPI, the pig had improved and was eating. A blood sample was collected and HC virus, $<10,000$ plaques/ml., was detected by FATCT. A total white blood cell (TWBC) count of 3,550 cmm. was determined for the sample.

At this point, it became obvious that this pig was experiencing something different than the usual chronic hog cholera infection. Periodic clinical observations and laboratory examinations were made as recorded in table 4. The HC virus titer in the blood remained high although the pig had periods of normal health. The numbers of HC virus particles in the blood samples collected on 51 and 82 DPI were so high that every cell on the coverslip cultures was fluorescing.

A necropsy was performed following death on 93 DPI, and the lesions observed were as follows: Marked congestion of the eyes; slight peripheral congestion of cervical, submaxillary, superficial inguinal, mesenteric, portal, and renal lymph nodes; fibrinous pericarditis; congestion, inflammation, and necrotic foci of the cecum and colon; and small ulcers in the mucosa of the stomach. Hog cholera virus was isolated from the tissues.

The HC virus in the blood specimens of this pig was found to be of high virulence when inoculated into a susceptible pig. Contact pigs placed in the same room with this pig while it was apparently in normal health developed acute HC infections and died.

The HC strains isolated from pigs older than a week of age included only one avirulent strain (table 3) and one strain of low virulence. The virus strain from herd 18892 was inoculated into three SPF pigs. Since one pig died 8 DPI, the virus strain was considered to be of high virulence although the other two pigs died 20 and 23 DPI.

The HC virus plaque counts per milliliter of spleen suspension were quite high except for the counts from the tissues of the 8-week old pigs from herd 17010.

The source of the virus was noteworthy in that six of the 11 strains were reported by the HC diagnosticians to be of vaccine origin. In some of the herds, 17010 and 18892, the sows were vaccinated during pregnancy. Of equal significance was the fact that five of the herds had no history of recent vaccination or exposure to vaccine virus.

Discussion

It is quite evident from the findings in table 1 and table 2 that HC has been responsible for many unexplained baby pig losses. The question is raised as to how many of these problems are caused by other viruses and will remain with us after the completion of the HC eradication program. Since the HC virus strains responsible seem to have characteristics of avirulence or low virulence, it is logical to suspect other viruses such as pseudorabies, swine influenza, and enteroviruses which usually produce mild, nonfatal disease in mature swine.

The failure to isolate pseudorabies virus from the specimens received was of importance. This virus will produce a cytopathic effect in 48 hours on the PK-15 cell cultures and is readily isolated without special culture procedures. If pseudorabies virus was present in the specimens from a herd included in the survey, it should have been picked up by the routine FATCT.

The etiology of Myoclonia congenita must be quite complex and include agents other than HC virus. The negative laboratory findings associated with herds in which no additonal evidence of disease was present may point to genetic, toxicologic, or nutritional factors.

It is suggested that the strain of HC virus which causes baby pig losses is of low virulence, causing a mild clinical illness in older pigs which may exist without detection unless laboratory examinations are made. Total depopulation is obviously the only sure way of eliminating a HC infection of this type.

The chronic course of illness resembling immunologic tolerance in the pig given the tissue suspension from aborted fetuses provides an indication that some HC strains have characteristics that favor the production of this abnormal state. It is questionable how long this pig would have survived among the vicissitudes of the average farm lot since good animal facilities provide an ideal environment. The concentration of virus in the blood of this pig was so high that it precluded the presence of antibody. Yet this was virus of high virulence when inoculated into other pigs. It is suggested that this pig was in a state of immunologic tolerance.

The ability of any virus to produce a persistent intrauterine infection may be associated with the special characteristics of low virulence or a facility to induce the state of immunologic tolerance. The title of this symposium directs us to consider fetal abnormalities in swine in addition to abortion and death. Certainly a newborn pig of normal appearance suffering from an immunologic tolerant infection contracted in the uterus and containing high titers of virus in its tissues is quite an abnormality.

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A PRELIMINARY REPORT ON IN UTERO TRANSMISSION OF HOG CHOLERA VIRUS IN PREGNANT SOWS[1]

By W. C. Stewart [2]

Summary

In utero transmission did not occur in the litters of 12 immune sows given virulent hog cholera virus. On the other hand, transmission occurred in four out of five and four out of four natural farrowed litters of susceptible sows given attenuated vaccine and a field strain of low virulence, respectively. Confirmation of in utero transmission was obtained in all but one litter by isolation of hog cholera virus employing the fluorescent antibody, tissue culture technique.

Vaccination of susceptible sows resulted in high mortality of baby pigs; approximately half were killed by the virus in utero. The majority of the live pigs were weak and died within 8 days. Mortality in mature swine, abortions, and early fetal arrest with death in utero resulted when susceptible sows were exposed to a strain of low virulence. Foremost was death of the baby pig in utero; prenatal death occurred in 19 out of 30.

Introduction

For more than a decade it has been known that vaccination of sows in early pregnancy with attenuated hog cholera (HC) virus resulted in abnormalities of newborn pigs[3]. The full impact of this discovery to the epizootiology of HC was not recognized, however, until recently. In England, pregnant sows exposed to field strains of HC virus were reported to serve as reservoirs or carriers for periods of several months.[4] Sows exposed to HC virus or vaccinated during pregnancy were reported to give birth to litters of infected pigs, thereby maintaining the virus on farm premises in the United States. [5]

In utero transmission (IUT) of HC virus was a phenomenon little understood in 1965; yet it appeared to be a problem of some consequence in farm herds, particularly units with continuous farrowing operations. Manifest at this time were serious baby pig losses resulting from apparent in utero infection by HC virus. Developmental studies were needed to provide information of importance to the HC eradiction program and to answer the following questions:

1. Did IUT occur irrespective of the immunologic status, that is, were immune as well as susceptible sows involved? Field reports had indicated involvement of vaccinated sows.
2. Following infection, how frequently did IUT occur?
3. Was IUT dependent on the stage of gestation?

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[1]The author acknowledges the assistance of R. A. Packer, members of his graduate committee, Iowa State University, and members of Diagnostic Virology.

[2]Animal Health Division, Agricultural Research Service, USDA, National Animal Disease Laboratory, Ames, Iowa.

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4. Was IUT more likely after exposure to cetain strains of HC virus, for example, virulent, low virulent, and attenuated?

5. Did pigs infected in utero become tolerant to the virus and serve as healthy carriers?

6. Was the virulence of a virus strain affected by IUT?

7. What were the effects on fetal development from IUT, that is, would abortions, mummified fetuses, or stillbirths occur?

Since it appeared that the answers to these questions were not available, a project was designed to study IUT of various HC virus strains in susceptible and immune sows at various stages of gestation.

Materials and Methods

Group 1, hog cholera immune sows.--Twelve sows previously vaccinated as weanlings with a modified live virus vaccine were given 1.0 ml. of virulent HC virus suspension that is, >10,000 plaques/ml. by the fluorescent antibody, tissue culture technique (FATCT). Preinoculation serums were tested for HC virus neutralizing antibodies by the fluorescent antibody, serum neutralization (FASN) test.[6] A sow was considered immune if it had a minimum titer of 1.2.[7] Gestation stages of the sows ranged from 13 to 88 days.

Group 2, hog cholera susceptible sows.--Eight sows originating from a nonvaccinated, HC free herd were given 2.0 ml. of a commercial tissue culture origin, modified live virus vaccine without anti-HC serum. Preinoculation serums were screened at the 1:4 dilution for HC virus neutralizing antibodies. A sow was considered susceptible if antibodies were not detected. Gestation stages of the sows ranged from 29 to 78 days.

Group 3, Hog cholera susceptible sows.--Ten sows from the same source as group 2 were given 2.0 ml. of HC virus suspension, that is, >10,000 plaques/ml. by the FATCT. The virus had been isolated from a field specimen; and on the basis of a single test pig inoculation, it was identified as a strain of low virulence. The sows were considered susceptible if HC virus neutralizing antibodies were not detected by the FASN test at the 1:4 dilution. Their gestation stages ranged from 33 to 84 days.

Following exposure to each strain of HC virus, all sows were observed daily for signs of illness, and temperatures were recorded for a period of 14 days.

Results

Group 1 sows.--Signs of illness were not apparent in the sows following inoculation with virulent virus, and the daily temperatures did not exceed 103° F.

A vigorous, healthy litter was farrowed by each sow. Litter sizes ranged from five to 11 with an average of 7.8 (table 1). Examination of 726 tissues from a total of 94 pigs did not produce a single isolation of HC virus.

Tests of preinoculation serums for HC virus neutralizing antibodies resulted in a mean titer of 1.8 and a range of 1.2 to 2.4. With the exception of two sows (1 and 9, table 1), an increase in titer was shown after challenge with virulent virus.

Group 2 sows.--Vaccination with modified live virus vaccine did not elicit adverse reactions, and only 2 sows had temperatures as high as 103.6 F.

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[6] Lee, L. R., Carbrey, E. A., Kresse, J. I., and Stewart, W. C. Fluorescent antibody, serum neutralization test for detection of hog cholera antibodies employing a plaque reduction technique. In Developmental studies conducted during fiscal year 1966, Diagnostic Services, National Animal Disease Laboratory. U.S. Dept. Agr., Agr. Res. Serv. ARS-91-63, pp. 16-22. 1967.

[7] The logarithm of the dilution of the serum that caused a 90 percent reduction in the plaque count (1000 plaques/ml.) of the test virus.

Table 1.--Group 1, immune sows given virulent HC virus

Sow No.	Number of days gestation	Pigs farrowed	Pig tissues examined by FATCT	HC virus serum neutralizing antibody titers[1]		Serums obtained days post-inoculation
				Preinoculation	Postinoculation	
		Number	Number			Number
1	88	5	34	2.4	2.4	52
2	82	6	43	1.8	3.0	56
3	81	10	71	2.4	3.0	56
4	79	7	55	1.2	2.4	52
5	52	9	71	1.8	2.4	76
6	46	7	55	1.8	2.4	90
7	39	11	87	1.8	3.0	83
8	37	9	71	1.8	2.4	90
9	37	8	64	2.4	2.4	90
10	34	8	64	1.2	3.0	90
11	30	6	48	1.2	2.4	103
12	13	8	63	1.8	2.4	110

[1] Expressed as the logarithm of the serum dilution which caused a 90 percent reduction in the plaque count (1,000 plaques/ml.) of the test virus.

To simulate field conditions, each sow was allowed to farrow naturally. Specimens were obtained from the baby pigs over a period of 0 to 24 days postfarrowing (DPF) and frozen until such time as they could be processed. In the case of healthy litters, single pigs were sacrificed on alternate days with few exceptions. The tissues were examined by the FATCT for HC virus as previously reported.[8] Tissues selected for examination were spleen, tonsil, mandibular lymph node, brain, lung, heart, kidney, and liver.

Serums were obtained from each dam after farrowing and tested for HC virus neutralizing antibodies by the FASN test.

Test pig inoculation.--A 30 lb. hysterectomy-derived specific-pathogen-free (SPF) pig inoculated with tissue suspension prepared from tissues of pigs farrowed by a susceptible sow of group 2.

Unlike the immune sows, farrowings were not normal. Five sows farrowed 46 offspring of which 23 were stillborn (table 2). Evident by their brownish discoloration, 11 of the stillborn pigs had been dead in utero for a time sufficient to permit autolysis and putrefaction. The bulk of the 23 pigs remaining alive were weak and died within several days. Hog cholera virus was detected by the FATCT in tissues of 27 pigs representing four litters (table 2). Tissues of two pigs surviving 7 and 8 days after farrowing were found positive by the FATCT. Virus not isolated from the litter of one sow, from those pigs that died in utero, and from one pig of a positive litter sacrificed 21 DPF.

Three sows vaccinated in the first trimester of pregnancy did not farrow and were not observed in estrus throughout the study. Uteri from the unproductive sows were collected at slaughter; however, careful examination failed to reveal any evidence of former pregnancy.

Serum neutralization tests of the serums collected from the sows 55 to 89 days post-vaccination resulted in titers ranging from 1.8 to 3.0 with an average of 2.25. (table 2).

[8] Carbrey, E. A., Stewart, W. C., Kresse, J. I., and Lee, L. R. Technical aspects of tissue culture fluorescent antibody technique. Proc. U.S. Livestock Sanit. Assoc. 60th Ann. Meeting, Lansing, Mich. pp. 487-500. 1965.

Table 2.--Group 2, susceptible sows given attenuated vaccine without anti-HC serum

Sow No.	Number of days gestation	Pigs farrowed	Pig tissues examined by FATCT	Virus isolations	Postinoculation serum antibody titers	Serums obtained days post-inoculation
		Number	Number	Number		Number
1	78	9	71	58	2.4	55
2	77	7	51	0	1.8	55
3	74	10	78	54	2.4	55
4	66	11	87	23	3.0	55
5	40	0	0	0	1.8	89
6	35	0	0	0	1.8	89
7	33	0	0	0	2.4	89
8	29	9	72	47	2.4	89

Table 3.--Group 3, susceptible sows given a HC virus strain of low virulence

Sow No.	Number of days gestation	Pigs farrowed	Pig tissues examined by FATCT	Virus isolations	Postinoculation serum antibody titers	Serums obtained days post-inoculation
		Number	Number	Number		Number
1	84	0	0	0	(1)	--
2	75	(2) 4	18	0	(1)	--
3	73	0	0	0	1.8	48
4	67	0	0	0	2.4	48
5	67	0	0	0	1.8	48
6	66	6	48	31	2.4	83
7	51	0	0	0	2.4	83
8	43	8	16	16	2.4	91
9	37	10	0	0	3.0	83
10	33	6	40	40	2.4	91

[1] Sow died.
[2] Aborted.

The SPF test pig inoculated to confirm isolation of virus by the FATCT and to demonstrate virulence of the isolate was observed to have inappetence 9 days postinoculation (DPI). Otherwise, the pig remained healthy, and subsequently it was found immune when challenged with virulent HC virus.

Group 3 sows.--Three sows were observed to be ill with elevated temperatures soon after infection with the strain of low virulence. One sow aborted 8 DPI and died later. Of the other sick sows, one subsequently died and one recovered. Seven sows were not observed to have clinical reactions.

In addition to the four fetuses aborted, four sows later farrowed 30 offspring of which 19 had been dead in utero. Eleven live pigs were weak and died within a 24-hour period. Hog cholera virus was isolated from all of the live pigs but not from four fetuses aborted in the 83d day of gestation and one litter consisting entirely of pigs dying in utero.

Four sows kept through the study did not farrow and, moreover, were never observed in estrus. The uterus of one sow dying 44 DPI was not found to contain fetuses. From the breeding dates furnished by the herdsman, it was concluded that the sows had never been pregnant.

Eight serums examined for HC virus neutralizing antibodies 48 to 91 DPI were found to have a mean titer of 2.3 and a range, 1.8 to 3.0.

Discussion

Failure to isolate HC virus by the FATCT from 94 offspring of immune sows given virulent HC virus indicated IUT did not occur. This conclusion was further supported by the absence of clinical illness in the sows and the farrowing of healthy, vigorous litters.

Inoculation of susceptible sows with attenuated vaccine and a strain of low virulence caused IUT. In the Group 2 sows, HC virus was detected by the FATCT in four out of five litters. On the basis of virus isolations and baby pig mortality, IUT was established in four out of four natural farrowed litters of the Group 3 sows.

In utero transmission occurred most often when the sows were infected with HC virus during the first and second trimesters of pregnancy. Little evidence was obtained for the occurrence of IUT in the final trimester. Only two sows infected in the early part of the last trimester farrowed, and of these, IUT was established for only one.

A common occurrence with IUT was the farrowing of stillborn and weak pigs. In the latter case, survival for 8 days was the maximum life-span. Infection of sows with a strain of low virulence resulted in one abortion, death of two sows, and high mortality in baby pigs. Prenatal deaths were accentuated by infection with the strain of low virulence.

Approximately 40 percent of the susceptible sows in Groups 2 and 3 did not farrow, yet none of the sows were observed in estrus. It was improbable that these sows could have had normal estrus cycles without being detected. If the sows were pregnant when exposed to HC virus, the silent estrus cycles may have resulted from IUT, fetal arrest, and subsequent resorption. The availability of a conclusive pregnancy test for swine would have eliminated the questionable status of the sows at the time of infection with HC virus.

If the results of a single test pig inoculation are considered, virulence of the vaccine strain was not increased by IUT.

DISCUSSION

Dr. Ray:

Do you get a positive FA from an animal that is carrying bovine virus diarrhea?

Dr. Carbrey:

Bovine virus diarrhea BVD antibodies will cross neutralize with hog cholera antibodies; this has been shown. The two viruses do cross-fluoresce, but pigs with antibodies against bovine virus diarrhea in Baker's work did not have antibodies against hog cholera, if I recall his work correctly. Perhaps I am not sure what you are getting at, but we have taken field

strains of bovine virus diarrhea and put them on our PK-15 cells, and they do not take off and produce fluorescence for us. We do have an adapted BVD strain which does produce fluorescence in the PK-15 cells. In Georgia tissues were taken from calves and processed as if they were hog cholera specimens. BVD viruses were not recovered from these specimens on PK-15 cells. Is that what you had in mind?

Dr. Ray:

This is actually what I had in mind. The new regulations require that we use antibody negative pigs in tests on hog cholera vaccines. We run into a lot of positive pigs that have never been vaccinated, pigs from where they don't vaccinate. These pigs show a positive reaction, positive antibodies in their blood. In checking these I was wondering if we might not be running into a situation where some of these positive pigs, positive on antibody examination, were actually not hog cholera. Nevertheless, we still have to eliminate them--the fact that they are positive FA pigs.

Dr. Carbrey:

Really we shouldn't do this. Dr. Combs--Dr. Shope in Minnesota did some work looking for antibodies, didn't he find about 3 percent BVD antibodies in pigs?

Dr. Combs:

That is correct.

Dr. Carbrey:

And 10 percent hog cholera antibodies in cows?

Dr. Combs:

Well, I am not willing to say how many he had in cattle. It's about that.

Dr. Dunne:

When I gave a paper on the diagnosis of hog cholera in Paris, there was a young fellow from the Netherlands who spent about 10 minutes trying to get people to say that bovine virus diarrhea would interfere with the test. I really wasn't too sure of my ground on this and I sidestepped him with all the finesse that I could establish and finally got off without actually saying positively one way or the other. After this he got up and stated that Brooksfield definitely stated that it did not interfere with the hog cholera test. He had questions like, "Would the serum in the tissue culture, which is used for the hog cholera test, interfere?" and so on. He had a whole string of questions like this, all of them which when analyzed got back to bovine virus diarrhea interfering with the test. How I got by without incriminating myself, I don't know. I wasn't real sure, but I remembered that Dr. Carbrey told me one time that they felt there was no interference. Also, I got this impression from two or three other places. I wasn't sure but it apparently is so, that the virus diarrhea antibody in the serum does not interfere with the test in tissue culture.

Dr. Gustafson:

Is Walter Myers here? I wanted to inquire about the paper reporting that the crossing occurred in response to a soluble antigen. Response to the soluble antigen would make possible some variations in whether or not the conjugate was made against the soluble antigen or whether it was made against the viral antigen. In some instances then if the conjugate was made against that, it would; if the conjugate was not made against that, it would not. This may be part of it, and I thought maybe Walter could straighten that out for us.

Dr. Carbrey:

You can use hog cholera conjugate to detect BVD infected cells just fine and vice versa. It just makes you feel a little better to use the proper one, that's all. Actually I would have to say that the BVD antibodies, if they were present in the pig, would not produce a cross neutralizing titer similar to the hog cholera virus. This is based on Dr. Baker's work. This does not eliminate the possibility that there are other viruses around that do have an antibody to share, or again, what is the status of the hog cholera strain in the population.

Dr. Dunne:

The point brought up about the presence of antibodies in cattle also brings up the possibility that serum used in the tissue culture diffusion test might contain antibody which would neutralize viruses if you used serum.

Dr. Carbrey:

We tried an FA test with cells that were actually growing in the serum that had a high BVD titer, as high as we could get. The only thing that happened was that the test worked fine; the cells grew better because they liked that serum.

Dr. Dunne:

Yes, but if you have hog cholera antibodies in the bovine serum as evidenced in the 4 or 10 percent, whatever it is.

Dr. Carbrey:

Yes, we have that.

Unidentified:

In the classification of viruses, serologic evidence of antigenicity is only one factor in the identification; does anyone have additional data, such as symmetry or ether sensitivity of the viruses?

Dr. Dunne:

This is all well catalogued. The hog cholera virus and the BVD virus apparently belong to the same class of myxoviruses. They are essentially the same size; they are ribonucleic acid (RNA) positive; they are ether sensitive. These are the catagories in which they place the classification.

FIFTH SESSION: M. J. Tillery,[1] Chairman

Paper No. 16

EFFECTS OF AUJESZKY'S DISEASE (PSEUDORABIES) IN PREGNANT SWINE[2]

By D. P. Gustafson,[3] J. R. Saunders,[4] and R. M. Claflin[3]

In North America, until 1962, Aujeszky's disease had been reported to cause sporadic deaths among baby pigs and rare recognizable disease among pigs weighing about 20 pounds (8, 14, 17, 18, 20). Evidence of the disease among mature swine was limited to the presence of specific virus-neutralizing antibodies. Beginning in late 1962 sickness and deaths among mature and immature swine caused by this disease have been observed (18) in an ever increasing number of locations in the United States. The severity of the disease has been less among mature swine in which case mortality in most instances is less than 10 percent. Infections among pregnant swine have been manifested in many cases not only by common signs of the disease but also by maceration of fetuses in utero, abortion, and early death of the newborn (18). The disease as it is now present resembles that which has been reported in northern Ireland, (5) England (7, 13), on the continent especially in Holland (1), Germany (2), Czechoslovakia (12), Hungary (4), and Yugoslavia (15). This report is concerned with experimental infections of pregnant sows and the effects of their progeny.

Materials

Sows and Gilts

Thirteen sows from the Veterinary Science Farm, Purdue University, were used as principals. Records on 23 consecutive farrowings among other sows served as control for the farrowing performance of the principals as shown in tables 1 and 2.

Virus

1262 "S" strain Aujeszky's disease virus (ADV) was used for exposure of the animals and for serum neutralization tests in cell culture (17). Virus pools were cell culture fluids of the fifth passage or lower from the original isolation. Virus in cell culture fluids titering $10^{5.5}$ tissue culture infective doses to a 50 percent end point ($tcid_{50}$) per ml. was used for exposure of the swine.

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Table 1.--Farrowing on research farm at Purdue University during the period the principals were obtained

Pigs farrowed	Litter number																
	1	2	3	4	5	6	7	8	9[1]	10	11	12	13	14	15	16	17
Alive	9	12	12	9	13	6	12	11	12	9	9	12	11	7	7	8	15
Dead	0	2	0	0	0	0	0	0	5	6	3	2	0	0	0	0	0
Macerated				2													

[1] Cesarean section.

Table 2.--Environmental control farrowing in isolation

Sow	Pigs farrowed		
	Live	Dead	Macerated
3677	9	1	0
3676	9	1	0
3736	9	1	0
3719	10	1	0
3489	5	0	0
3529	7	0	0

Cell Cultures

Monkey kidney cells (LLC-MK) (11) or pig kidney cells (PK-15)[5] were used in virus titration, virus isolation, and serum neutralization tests.

Cell Culture Medium

The medium for cell cultures was Eagle's basal medium (EBM) with 2 percent fetal calf serum and 100 units of penicillin and 0.1 mg. streptomycin per ml.

Isolation Facilities

Rooms for isolation of the swine were in a building specifically designed for the purpose. Each room has an air intake and exhaust and an anteroom entrance. As an added precaution, persons entering each room change outer garments and footwear. Feed was supplied to each room from the floor above through a chute, each from a closed hopper.

[5]American Type Culture Collection Registry of Animal Cell Lines Certified by the Culture Collection Committee. First edition 1964, including the first supplement (1965) and the second supplement (1967). American Type Culture Collection Cell Repository, 12301 Parklawn Drive, Rockville, Md. 20852.

Methods

Exposure of Swine to ADV

Virus was administered intranasally to all principals, in the manner previously described (19).

Quantitative Procedures

Total white blood cell (w.b.c.) and, in the first experiment, differential w.b.c. counts were made from blood before exposure and at various times after exposure.

Serum samples obtained before exposure to ADV and at various times after were tested for the presence of neutralizing antibodies as previously described (19).

Rectal temperatures were taken daily as an aid in following the course of reaction to exposure to the virus. Observations were also made of appetites and the character of eliminations in exposed swine.

Attempts to Isolate Virus

In Experiment A, samples of brain, liver, spleen, and lung were removed from 43 live fetuses and from three macerated fetuses. The samples were tested in cell culture for the presence of virus. Each tissue sample from individual fetuses was ground in sand with mortar and pestle; then a small amount of balanced salt solution (BSS) was admixed with each. Equal amounts of supernatant fluid from each of four or five centrifuged samples of each tested type of tissue from a like number of fetuses from a single sow were pooled and filtered through an 0.45 μ cellulose ester filter. Subculturable swine kidney cells (PK-15) in 16 x 150 mm. glass tubes were exposed to 0.1 ml. of the pooled and filtered extracts from ground tissue samples. Four tubes were used for each sample. When pooled samples were found to contain the virus, the individual samples forming such were applied separately to similar cultures to identify the specific samples that were the source of virus found in the pooled samples. Similarly, efforts were made to isolate the virus from samples of placenta and brain from each of sows A, B, and F.

In experiment B, rectal and oral swabs from six pigs from sow 5 were soaked in 2.0 ml. of Hanks' BBS with 200 units of penicillin and 0.2 mg. streptomycin. The swabs were removed and the fluid centrifuged at 5° C. at 2,500 r.p.m. for 30 min. Three drops of the supernatant fluid from each sample was added to each of three tubes of MK_2 cells.

Experimental Procedures

Experiment A. Exposure of Pregnant Sows to ADV and Examination of Fetuses in utero

Six pregnant sows from the Veterinary Science Farm, Purdue University, were bred during a 2-month period by a single boar. A few days before exposure to ADV, two sows each were put into three isolation rooms. Seventeen sows due to farrow over the same period as the principals (table 1) served as environmental controls on the farm and six other pregnant sows (table 2) were placed in other isolation units in the same building housing the principals. None of the controls were exposed to ADV.

Blood samples for leukocyte counts and serum neutralization tests were collected from the principals daily for 2 days before exposure and for 10 days after exposure as previously outlined (18). Rectal temperatures were taken twice daily before and after exposure. All six were exposed to 2.0 ml. of ADV on the same day. Sows A and B were exposed at 93 days of pregnancy; C and D were at 72 and 62 days, respectively; and E and F were each exposed at 45 days of pregnancy. The swine were maintained in isolation until death or until killed approximately 2 days before an estimated farrowing date. Before exposure and 2 weeks after exposure, blood samples were obtained to test for virus-neutralizing antibodies in the serum. At death, or when killed by electrocution near term, the sows were necropsied and uteruses were removed as quickly as possible. The uteruses were incised for gross examination of fetuses present. Various tissues (spleen, liver, lung, kidney, and brain) both maternal and fetal, as well as placenta, were collected for histopathologic examination. In addition to those from the six sows tissues were examined from those fetuses (65 total--43 live and 22 macerated) that were at all suitable for such an examination.

Experiment B. Exposure of Pregnant Sows to ADV and Evaluation of Results During Gestation and Post Parturition

Seven pregnant sows from the Veterinary Science Farm, Purdue University, were transferred to the isolation facilities. Two were placed in each of three rooms and one in a fourth room. Serum samples for tests for virus-neutralizing antibodies were obtained from blood samples taken from the sows during a 5-day preexposure period. Each was exposed to 2.0 ml. of ADV. The clinical response was observed, and rectal temperatures were obtained daily. Following parturition of each of two sows (11A and 11B), blood samples were obtained from four and five baby pigs, respectively, before suckling colostrum, to test for the presence of virus-neutralizing antibodies in the serum. Serum samples were obtained from pigs after weaning and from sows for the same purpose after parturition.

Nasal and rectal swabs were obtained from sow 5, 23 days after exposure to the virus and tested in cell cultures for the presence of virus. Two days after sow 10 A farrowed, an extract of the brain of one of the surviving piglets was tested in cell cultures for the presence of virus.

Tissues for histopathologic examination were obtained from sows 10B and 11A which were killed at 1 and 57 days post-parturition, respectively.

Results

Experiment A. Exposure of Pregnant Sows to ADV and Examination of Fetuses in utero

Clinical observations.--The clinical response of the six sows to intranasal exposure with ADV is shown in table 3. Sow C developed a fatal encephalitis; clinical signs were similar to those of feeder pigs with natural infection (4). The remaining five principals developed mild disease, maximum rectal temperatures were found to be 105° F; constipation and anorexia were present. Recovery in these animals was complete, generally by 7 days postexposure. The sow that died became progressively depressed, suffered intermittent convulsions, ultimately becoming prostrate, and comatose before death.

Antibody response.--Virus neutralizing antibodies of at least 1:2 against 50 $tcid_{50}$ of ADV were found in the serum of all 5 living principals when tested at 3 weeks after exposure. All were negative before exposure. Furthermore, all swine, more than 100, from the source herd that have been tested for virus-neutralizing antibodies in the serum have been found to be negative.

Table 3.--Response of pregnant swine to intranasal exposure to Aujeszky's virus

Sow No.	Fecund days to virus	Signs				Blood leukocyte changes[1]					
		Body temp.		Anorex. Days	Encephalitic signs in days	Polymorph		Eosinophils		Total	
		Max.	Days			Shift	Day	Count	Day	count	Day
A	93	104.4	3-6	3-6	--	Mild Left	4	+sl	4	sl+	4
B	93	104.	3-6	3-6	--	Mild Left	4	+sl	2	--	--
C	72	107.8	3-7	3-6	3-7 (died)	Mild Left	4	+	2	--	--
D	62	104	3-7	3-7	--	N	--	N	--	--	2
E	45	103.6	4-8	5-8	--	N	--	+	2	--	--
F	45	105	3-8	3-9	--	N	--	N	--	sl-	2

[1] N = No change.

Table 4.--Fetuses obtained from A.D. Virus-Infected sows and controls

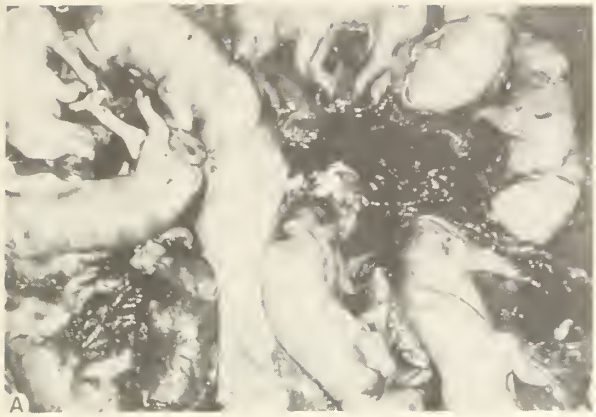
Animal	Age of gestation		Number of fetuses	
	At exposure	When killed	Live	Macerated
A	93	112	9	1
B	93	112	9	2
C	72	[1] 79	10	0
D	62	105	10	0
E	45	112	2	10
F	45	109	3	9
CONTROLS (17)	--	--	207	[2] 9

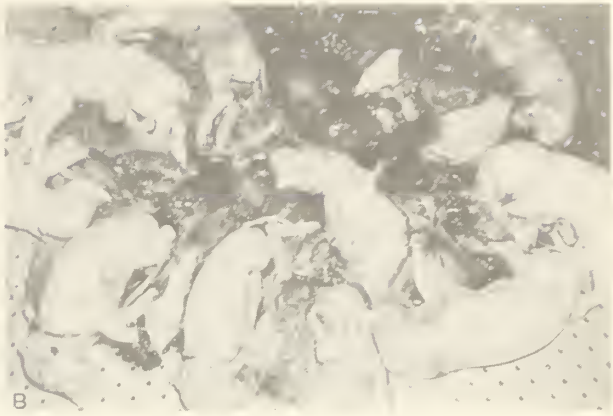
[1] Died 7 Days post-exposure.
[2] Dead, not mummified.

Control farrowings.--Table 1 shows consecutive environmental control farrowings on the Veterinary Science Farm excluding those that were brought to the campus isolation facilities for use in these and other studies. In addition, as shown in table 2 are six consecutive farrowings in the isolation facilities of sows brought from the farm as environmental controls for the conditions of isolation.

Gross observations on uteruses and contents.--In table 4 the day of gestation on which each of the principles were exposed to the virus is shown together with the results at the time estimated to be within 2 days of farrowing. It was at this latter point that the sows were killed so that observations of the intrauterine conditions could be made. (See fig. 1.) Gross pathologic findings in the sows were found to be confined to the uterus and the fetuses in all five which recovered from exposure to ADV. The sow (C) that died at 7 days had gross changes of minor vascular engorgements of the viscera in addition to severe non-suppurative meningitis. In the uteri of sows A, B, E, and F the fetal sacs that contained the macerated fetuses were discolored--a chololate color--indicating reduction of heme.

There was a wide range of sizes among the 10 and nine macerated fetuses, respectively, in the uteruses of sows E and F. However, no evidence of malformation among them was observed.





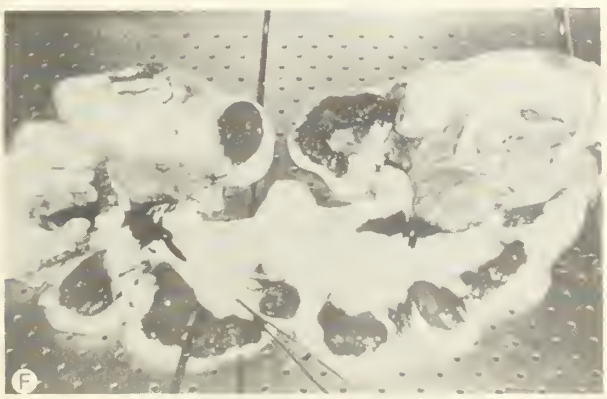


Figure 1.--Uteruses and fetuses from sows of Experiment A. Each photograph is marked with a letter (A, B, D, E, and F) designating the sow it represents. (Sow C died 7 days postexposure.)

Histopathologic observation.--Histopathologic changes suggesting congenital malformations were not seen in the case of fetuses that were firm and fresh and that were alive at the time the sows were killed. Changes in macerated fetuses were most difficult to interpret because of tissue destruction.

Changes observed in the central nervous systems of these sows ranged from only minor postmortem changes to a mild, apparently nonspecific, nonsuppurative meningoencephalitis. Widely scattered areas containing slightly increased numbers of glial cells were observed in the cerebrums and midbrains of four sows. Only very rarely were these assembled in small nodules. Perivascular accumulations of round cells two or three layers deep were observed occasionally in sections from two sows.

Random histologic observations of lymph nodes, kidney, lung, liver, spleen, and placenta failed to reveal significant changes or changes referable to ADV in either sows or fetuses. Histologic examination of representative sections of a wide variety of locations in the CNS of fetuses both living and dead at the time of necropsy failed to reveal lesions referable to ADV infection. Autolytic changes obscured most detail in the dead fetuses and among those surviving to necropsy only very widely scattered small glial accumulations were observed near the meninges in cerebellar sections which are commonly seen in swine fetuses. Similarly, a few instances of neuronophagia were seen, and a very few blood vessels were surrounded by two or three layers of cells.

Virus isolations.--Table 5 presents the results of efforts to isolate the virus from tissue of fetuses. Virus was not isolated from samples of placenta or brain from sows A, B, and F.

Experiment B. Exposure of Pregnant Sows to ADV and Evaluation of Results During Gestation and Post-parturition

Clinical observation.--Table 6 presents a summary of the clinical reactions to ADV of seven sows that were permitted to continue their pregnancy to term. None in this group died as a result of the infection. As in experimental infections previously reported, the thermal reaction begins at the third or fourth day accompanied by malaise, constipation, and anorexia. In addition, the farrowing statistics are presented. Sow 8A aborted an unknown number of fetuses during the night between the sixth and seventh days. Those found were approximately 2 inches in length. Sow 10B gave birth to 14 macerated fetuses 21 days after the calculated farrowing date. The other five sows farrowed without incident.

Serological evaluation of neutralizing antibody response.--Serum samples from each of the sows taken before exposure to the virus were negative. Those taken from the sows at various times after exposure were all positive as shown in table 7.

Antibodies were not demonstrated in the sera of five colostrum deprived pigs of sow 11A or four similar pigs of sow 11B. Serum samples from the same pigs of sow 11B 16 days later were tested and one of five neutralized virus at 1:2 dilution. All other serum samples from pigs were

Table 5.--Isolation of pseudorabies virus from fetuses

Tissue	No. Positive	No. Negative	Source	
			Fetus	Sow
Brain	1	46	2	B
Spleen	1	46	3	B
Lung	1	46	3	B
Liver	0	46	--	--

Table 6.--Response of pregnant swine to Aujeszky's disease

Isolation Unit	Animal No.	Days Pregnant at exposure	Signs at days postexposure		Farrowing results		
			Hyper-thermia	Anorexia	Live	Dead	Macer-ated
12	5	103	3 to 8	3 to 8	6	2	0
9	8A	35	3 to 6	3 to 13	ABORTED AT 7 DAYS		
	8B	30	3 to 7	3 to 15	9	0	0
21	11A	70	4 to 9	4 to 9	16	2	0
	11B	50	4 to 6	4 to 10	13	2	0
7	10A	86	4 to 7	4 to 10	7	1	1
	10B	85	4 to 9	4 to 10	0	0	14

Table 7.--Titers of virus neutralizing antibody in serum at various days after exposure to ADV

Sow	Day - Titer
5	23 - 1:8
8A	146 - 1:32, 252 - 1:8
8B	144 - 1:32, 252 - 1:32
10A	31 - 1:16, 123 - 1:16, 148 - 1:16
10B	31 - 1:32
11A	42 - 1:4
11B	42 - 1:8

negative. These samples were taken from one of six of sow 5 at weaning, six of six from sow 8B at weaning, and six of eight from sow 10A at 104 days of age which was 47 days after weaning.

Sow 8A having aborted at 7 days postexposure was artificially rebred and farrowed 16 pigs which was 254 days after exposure to ADV. Twelve were weaned. As shown in table 7, the sow had a titer of at least 1:8 at 252 days postexposure. Six pigs tested for virus-neutralizing antibodies were negative at 56 days of age.

Sows 8B, 10A, and 11B have been rebred after 6 months of isolation and are pregnant at time of writing.

Virus isolations.--Virus was not isolated from nasal and rectal swabs obtained from sow 5 at 23 days postexposure nor from similar materials from her six pigs. Virus was not found in brain extract of a 2-day-old pig from sow 10A.

Histopathologic observations.--Sections from the uterus, cervix, pancreas, lung, adrenal, kidney, spleen, liver, heart, mandibular lymph nodes, salivary glands, and tonsils from sow 10B (1 day post partum) were examined.

The edema, congestion, and small hemorrhages expected in an immediately postpartum cervix were noted. Numerous small epithelial ulcers were observed with craters filled with neutrophils, lymphocytes, and tissue debris. The submucosa contained a thin layer of neutrophils

just beneath the epithelium and the submucosa a fairly generous sprinkling of lymphocytes, neutrophils, eosinophils, and macrophages.

The epithelium of the uterus while not ulcerated did contain microcyts filled largely with neutrophils which occupied the sites of submucosal gland duct openings. The submucosa contained numerous microabscesses just under the mucosa and rather heavy scattering of lymphocytes, neutrophils, eosinophils, and macrophages in deeper layers. Degeneration of submucodal glands and ducts, edema, congestion, and hemorrhage suggestive of the postpartum state were also observed.

The only observation worthy of note in the other tissues was the considerable number of eosinophils in the mandibular lymph node and tonsils.

Sections of the uterus, cervix, cerebrum, and cerebellum were examined from sow 11A (57 days postpartum). Other than a fine scattering of lymphocytes in the submucosa, no abnormalities of the uterus and cervix were noted. No abnormalities clearly separable from processing artifacts were observed in sections of cerebrum and cerebellum.

Inclusion bodies were not observed in material examined in this experiment.

Discussion

The resulting death and maceration of fetuses in pregnant sows exposed to AD virus parallels that which has occurred in natural infections (7, 12, 13, 17). In general, a greater number of fetuses were killed and macerated when infection occurred during early stages of gestation rather than later stages. Such findings are compatible with other viruses that kill or interfere with normal development of the fetuses as in the case of partly attenuated hog cholera virus in swine (3, 6, 9) or with rubella virus in humans (22). However, unlike hog cholera or rubella viruses the AD virus was not found to cause congenital malformation. Because of the wide range of sizes among the macerated fetuses found in the uteruses of sows E and F, it seems reasonable to assume that the fetuses die at different times.

The recovery of virus only from fetuses in the uterus of sow B, 19 days after exposure but not in any of the tissues of any of the other fetuses tested suggests that the virus was not present much beyond 19 days after infection of the sow or that it was not in a recoverable form at later times. The position of live fetuses situated between macerated ones in the uterus indicated that they are selectively affected rather than sequentially or uniformly affected.

Demonstration of virus in a fetus and the deaths of many indicates that the virus may be transferred from the sow to the fetus. The low rate of success in finding virus in nasal and rectal swabs, fetal tissues, and placenta suggests that other tissue such as tonsillar tissue (15) might have been more suitable, that the virus is quite labile under these conditions, or that it disappears in about 3 weeks as the infection in the sow subsides.

It is also interesting to note that all of the progenies tested, with one exception, were devoid of measurable virus neutralizing antibody in their serum within a short period after being farrowed. At weaning or later no antibodies have been found in progeny of exposed sows, and they are susceptible to the disease. It had been considered within the realm of possibility that some of those born alive would be found to be shedders of the virus with or without the continuing presence of antibody as has been reported for lymphocytic choriomeningitis of mice and hog cholera (3, 10). Such has not been the case. That natural conditions of rebreeding would provide an additional chance for exacerbation has been considered. Thus far little evidence has been obtained to support this thought. Reports by some (12) that recovered swine may become carriers and intermittent shedders was not confirmed. It is interesting to note that lesions referable to ADV were not observed even in those fetuses from which virus was isolated.

Summary

Thirteen sows were exposed to Aujeszky's (pseudorabies) virus at various times in pregnancy, ranging from 30 to 103 days. Death, abortion, and delayed farrowing of macerated fetuses occurred among them. Five sows were killed just before term and the contents of their uteruses, which contained 43 live and 22 macerated fetuses, were examined. Virus was isolated from three of eight tissue samples from two fetuses of a sow exposed 19 days previously but not from 164 tissue samples of 41 other viable fetuses. Virus was not found in 12 tissue samples from three macerated fetuses. Histopathological findings of the fetuses were essentially negative. Findings in the sows suggested resolution of infection responses in the central nervous system.

Five of seven sows not killed farrowed 51 live and seven dead pigs. Colostrum deprived piglets were without neutralizing antibodies. Other piglets had low antibody titers and were susceptible to contact exposure at 8 weeks of age. Virus isolation efforts from these sows and piglets at various times were negative. Four sows have been rebred and one has farrowed normal susceptible young.

Conclusions

Intranasal exposure of pregnant sows at any stage of pregnancy may cause death of the sows, or death of fetuses with subsequent premature or delayed expulsion from the uterus.

The virus was not found to cause congenital anomalies.

The breeding value of four sows, one of which had aborted, was not impaired.

The offspring surviving through weaning were found to be susceptible to the virus and without measurable virus neutralizing antibodies in their serum.

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DISCUSSION

Dr. Morehouse:

Do you feel that your most likely route of exposure in the field is intranasal? This works experimentally, but is this the way it gets around in the field?

Dr. Gustafson:

Yes, I believe so, We found that other routes were possible. We can get a response in some animals by intragastric exposure. And as Dr. Nutte pointed out and others have demonstrated, we can get response by intramuscular inoculation, which results in a paralyzed limb. Subcutaneous inoculations (which we did not do but others have reported) result in a milder infection than that which occurs with intranasal exposure. We exposed pigs intratracheally and gastrically. I think because of the activities of swine there would certainly be room to assume that this is a very good way for pigs to become infected.

Dr. Stewart:

Dr. Gustafson, correct me if I am wrong. You isolated viruses from only two animals?

Dr. Gustafson:

Yes, two animals. So we believe that the virus can be transferred, and death of the embryos does not occur necessarily because of destruction of the circulation in the dam. It seems since we can find a virus, even in a few embryos, that the virus is actually transferred to those embryos that are killed.

Dr. Stewart:

At what stage of gestation is this sow in when exposed?

Dr. Gustafson:

I think about 35 days. It was one of the ones that were exposed early.

Dr. Dunne:

I would like for Dr. Ray to comment on pseudorabies as we had a lot of interesting experiences a few years ago.

Dr. Ray:

I think this virus is a lot more stable than some people give it credit for. I remember the first pig brain that I ever had with this virus in it. I kept this rabbit's brain in an ordinary refrigerator ice cube tray for about a year and a half and it was still pathogenic for rabbits. In this time it had thawed out two or three times, and it had become a little odoriferous. It would still kill rabbits, not from putrefactive organisms either. I got afraid to inject it, so I scarified the rabbits and rubbed the brain on.

I would like to say something, a little bit out of line here, but the fact that cattle management is one of the things that you have been confronted with a lot of times. Not more than one bovine animal out of four, that I have seen with this infection, has developed itch. So, if you find cattle dying under peculiar circumstances like getting hung in a feed-bunk or tangled up in wire fence and dying under peculair circumstances, check them for pseudorabies virus. That may be

the problem. You don't have to have cattle that have been exposed to pigs to have this happen. Not more than one out of four showed the "mad itch" syndrome, in which we found pseudorabies virus. I think that would be all I have to offer. I appreciate the fact that you got to get your eye peeled to pick this up in the field and I'm sure I have missed it many, many times. Bill Sippel started finding it common in south Georgia, and they still find it common in Florida. I'm glad to find they are picking it up in this part of the country and recognizing it.

Now I would like to say something about the antiserum that Dr. Shope tested. Most of the hog cholera serum produced in the midwest was produced in garbage-fed hogs. That would mean that these animals were exposed to garbage accumulated in these old garbage dumps. Chances are they had a much better chance of developing the disease and going through the disease unobserved than those on farms would have. That could account for the fact that a lot of this serum produced in the Midwest would be more apt to have antibodies in it than serum from an ordinary farm hog.

Dr. Gustafson:

All the textbooks related that swine infected with pseudorabies virus do not rub and do not itch. Some swine do, and they'll rub the skin right off their ears and heads.

Dr. Ray:

If this infection invades a farm at farrowing time and the herd is susceptible, you will have up to 75 percent loss in those baby pigs. In those instances, they are dying in a period that we usually associate with TGE. I don't believe you'd get them confused though, because of nervous manifestations. Some will live if you could feed them, but they are so sensitive and get such a high reflex, they can't even touch the teat without flying back.

Dr. Dunne:

I can verify that these cattle don't all itch. I remember one time I had just gotten out of school, when one of the first things I did was to work with Dr. Ray. We had a cow with colic and I was called upon to go out and treat this animal for colic. I assembled the speculum and all the equipment, the stomach tube, the pail, and the ingredients you put into it. I was getting all ready to put this into the animal when it up and died. I couldn't help thinking about what would have happened if I had gotten the tube into it first and started putting something into it before it died. At any rate, this animal died and later we took tissues. It had an edema around the omasum. We took some of this fluid and injected it into rabbits. I don't know why we did this, but we got pseudorabies reaction out of it. The rabbit itched and finally died on the spot. And the other thing I just wanted to mention was these serum cattle, on occasion, developed pseudorabies by the pigs gnawing at the vulva. I have a whole series of tissues that we took at one time of an animal that got infected from the nose of the pig gnawing on the vulvar parts of the animal.

Dr. Morehouse:

Since we are on swine diseases, I just wanted to mention that we have a lot of rabies in Missouri. This is one of my reasons for the question to Dr. Gustafson about exposure. We had a situation just recently in which a man lost over half of a herd of 40 swine with rabies. The interesting thing about this was that undoubtedly, the original exposure came from wildlife. When these animals started to die, the man did pen them up, but the rest continued to die over a period of about 2 months. They didn't seem to be biting each other frequently. Of course, this is a thing that adds a little confusion to problems of this type.

Dr. Gustafson:

Along the lines of the efforts to produce carrier animals and to uncover the latent infection in pseudorabies, surely the incidence and the occurrence of pseudorabies among swine in the country suggests that this type of thing does occur. I am thinking of one or two experiments that have been done with regard to pseudorabies virus in cell culture and with antiserum that is used in the serum neutralization test such as the one I showed. When several cell passages have been made with continued use of antiserum, the cell cultures remained normal. When the antiserum was removed from the medium, several days later, the virus then began to appear. This is a kind of a virus, a double strand, DNA, herpes virus, in which the cell transfer of the viruses is characteristic of this type of agent. One of the things that probably is occurring, among the swine in this country that have been exposed, is that periodically and intermittently they shed the virus. The circumstances under which they do this we have not been able to duplicate. I believe that it is possible that many of these animals that we expose could do this. We are looking for this in subsequent pregnancies to see if we can provide this circumstance.

Paper No. 17

EPIZOOTIC SWINE STILLBIRTH CAUSED BY JAPANESE ENCEPHALITIS VIRUS

By Tomiaki Morimoto[1]

Japanese encephalitis is a mosquito-borne viral disease which occurs in Japan and several Far East countries. The disease has a summer-fall seasonal incidence in Japan and has assumed epidemic form for many years. Culex tritaeniorhynchus is one of the principal vectors of the disease.

Humans and horses are the most susceptible hosts manifesting the symptoms of encephalitis following natural infection by this virus. Concomitantly with the epidemics among humans and horses, a large number of swine stillbirths occurred in many parts of Japan. The dams were symptomless during the pregnancy but produced stillborn or abnormal progeny. A high incidence of inapparent infection with Japanese encephalitis virus was demonstrated among swine by serological surveys.

Histopathological examination of a number of cases of stillborn fetuses revealed lesions of nonpurulent encephalitis. From the brain of a typical case of stillborn fetuses, several investigators have isolated a virus identified as Japanese encephalitis virus. Shimizu and his associates (32) inoculated the virus of Japanese encephalitis into pregnant swine and successfully produced stillbirths similar to those observed in natural cases.

This paper deals with epizootiological and etiological aspects of swine stillbirth commonly observed in Japan during the late summer and early fall.

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Properties of the Virus

Japanese encephalitis virus is one of the riboneucleic acid (RNA) containing agents (7, 22,) classified as Group B arbovirus, based on its antigenic properties (2). The size of the infective particle is relatively small, having a diameter of 38 mμ with a dense center and a distinct membrane, as observed in ultrathin tissue by electron microscopy.[2] The infective particle is sensitive to the action of ethyl ether, deoxycholate and lipases as well as some proteolytic enzymes (40).

The virus is cultivable in various tissue culture cells with or without manifesting cytopathic effect (CPE). Chick embryo cell cultures are used mainly for plaque assay of the virus (23, 29). Hamster kidney (11, 24), porcine kidney (16), and stable line of porcine kidney cells (8) are used for both CPE and plaques.

The virus has a relatively wide range of pathogenicity for various species of animals. Experimentally, a variety of mammalian hosts can develop a fatal encephalitis following intracerebral inoculation. Adult chickens are relatively resistant to infection with the virus but some domestic and wild birds are found to be susceptible. A high incidence of inapparent infection with the virus has been demonstrated in both human and animals by antibody surveys.

Japanese Encephalitis Virus Infection Among Swine

Japanese encephalitis virus (JEV) is known to be transmitted by the bite of the mosquito. The disease has a seasonal incidence in Japan. When sera were collected from pigs during the nonepizootic season, such as in winter, the distribution of antibody to JEV among swine was strictly dependent on their age. Figure 1 shows one of the results obtained by Kurata (14). These sera were collected throughout Japan during March 1965, and a total of 4,542 specimens were tested for their presence of hemagglutination-inhibition (HI)-antibody to JEV. High percentage of the antibody was observed in pigs both under 3 months of age and over 7 months of age. It is suggested that the former pigs might have received residual maternal antibody through the colostrum of dams, but the latter pigs developed antibodies through natural infection during the summer months of the previous year. The remaining pigs, which were between 3 months and 6 months of age, were born and raised during the off season of JEV epizootic and had few antibodies to JEV. Such antibody negative pigs became positive during the following summer season. Figure 2 shows the seasonal incidence of JEV infection in swine as determined by serological survey.[3] Serum specimens were collected periodically from pigs at a slaughterhouse and tested for the presence of HI-antibody to JEV. The sera collected in April and May had no antibodies to JEV. Frequency of the antibody positive serum increased markedly between the end of June and early August. Thereafter almost all pigs had antibodies to JEV. When the summer season was over, the percentage of pigs showing antibodies decreased gradually.

The appearance of antibodies to JEV in pigs were correlated with the occurrence of JEV-infected mosquitoes (13, 26, 39).[4] Figure 3 shows the relationship as observed in Fukuoka Prefective in 1964 by Otsuka and others (26). The virus was not isolated from the mosquitoes collected on June 12 but were isolated from the specimen collected on June 29. Subsequently, the virus was repeatedly isolated from specimens obtained throughout July. Concomitantly with the occurrence of JEV-infected mosquitoes, antibodies to JEV were developed in the sentinel pigs.

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[2] Higashi, N., Inoue, Y., Matsumoto, A., and Fujiwara, E. Electron microscopic studies on Japanese encephalitis virus grown in tissue culture cells. Eleventh Annual Meeting of the Japanese Society for Virologist. 1963.

[3] S. Otsuka. Personal communication. 1966.

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Figure 1.--Distribution of HI-antiboy to JEV among swine at nonepidemic season, March 1965. (Adapted from Kurata, 14.)

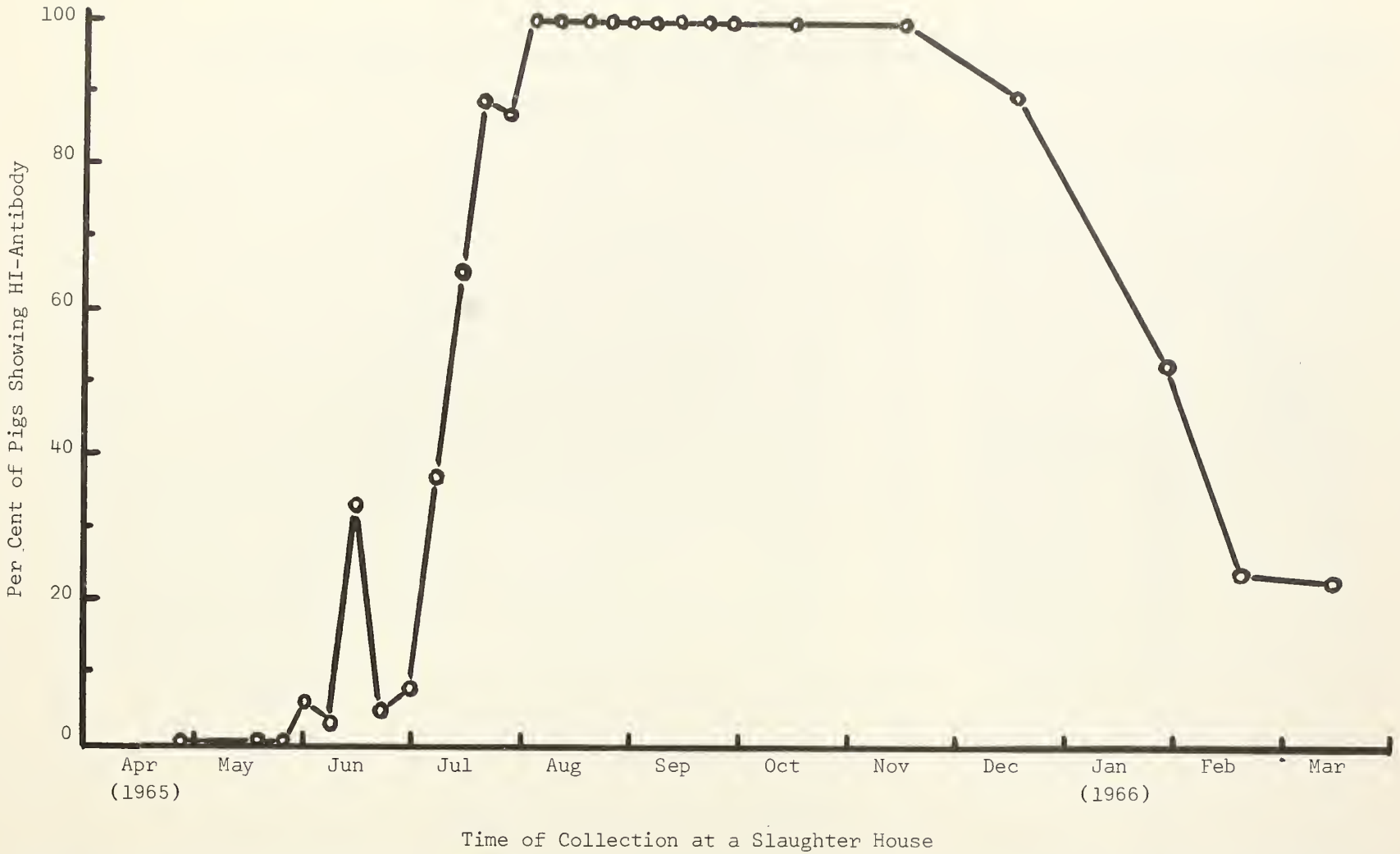

Figure 2.--Seasonal incidence of Japanese encephalitis virus infection among swine, Fukuoka Prefecture, 1965-66. (Adapted from S. Otsuka, Fukuoka Prefecture Institute of Hygiene, Fukuoka, Japan, 1966.)

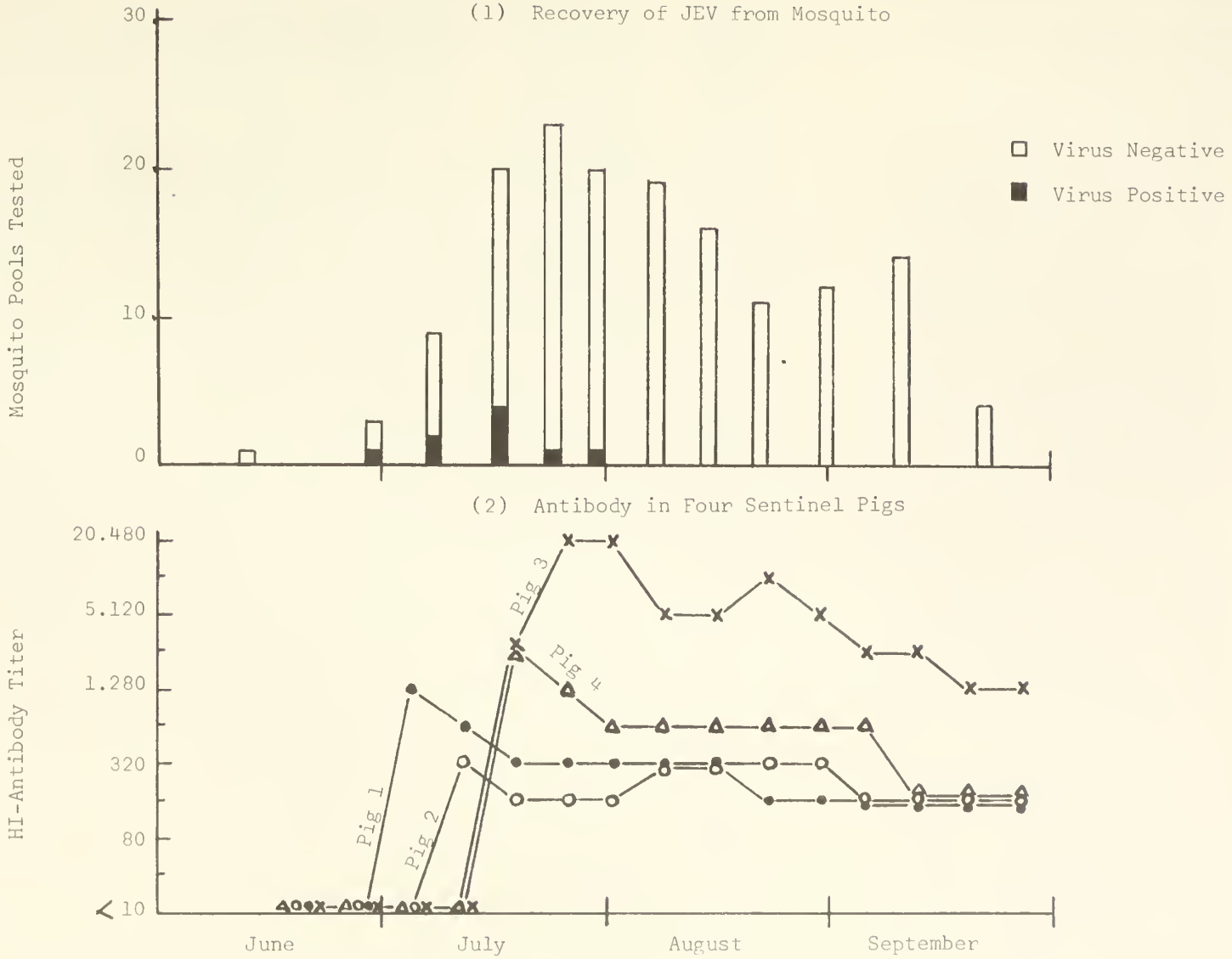

Figure 3.--Correlation between occurrence of JEV-infected mosquito and development of HI-antibody to JEV in pigs. (Adapted from Otsuka and coworkers, 26.)

The first development of the antibodies was observed in one of the four sentinel pigs on July 4, 1 week after the first detection of the virus in mosquitoes. On July 11, antibodies were detected in a second sentinel pig. The remaining two pigs became positive on July 18.

It was important to know the seasonal occurrence of JEV infection among swine, because the pig was one of the hosts most likely to serve as an amplifier of the infection which was passed by the mosquito in a cycle involving pigs and man (13, 39). A nationwide serological survey of pigs during the summer season was conducted by the Ministry of Agriculture and Forestry and Ministry of Welfare for the purpose of predicting JEV outbreak in the human population.

Occurrence of JEV-infected swine was first recognized in southern Japan at the end of June. Subsequently, the disease spread throughout the Nation, in a northeasterly direction, throughout the month of August, until all but the northern parts had infected pigs.

A similar tendency and pattern of geographical and seasonal occurrence of JEV infection among swine was observed in both 1965 and 1966.

Epizootic and Etiologic Aspects of Stillbirths

In 1947 and 1948, when severe outbreaks of Japanese encephalitis occurred among horses and humans, a large number of cases of stillbirth occurred among swine in Japan. Since then, these have recurred annually, especially in the late summer and early fall. Great efforts were made to clarify the etiological and epizootiological features of this disease by Japanese investigators.

Considerable data have been accumulated on the epizootiology of this disease following the epizootic of 1947 (1, 6, 20, 30, 31, 36, 41, 42). Table 1 shows the seasonal distribution of swine stillbirths that occurred in the Nagano Prefecture in 1951 (1). Of 247 cases, more than 90 percent occurred between August and November. The remaining few cases were distributed throughout the year. Generally, only 4.3 to 17.0 percent of the herds were involved but in certain districts as many as 50 to 83 percent of pregnant sows produced stillborn fetuses.

Breeding data of those 247 cases of sows that delivered stillborn fetuses are shown in table 2. About one-third of those cases of stillbirths were seen in the primiparous gilts that had been bred between May and July. It was suspected that most of these gilts must not have had antibodies to JEV when they were bred, presumably they were born after the JEV epidemic of the previous year. As mentioned before, the time of occurrence of JEV infection among swine was between the end of June and the end of August. This suggested that such gilts were infected with Japanese encephalitis virus during early pregnancy.

The gestation periods of those dams were variable. Of them, 33.7 percent farrowed normally (between 113 and 115 days after breeding) and 89.2 percent were distributed between the periods of 107 and 121 days. In general, there was a tendency toward prolonged gestation periods rather than shorter ones.

Table 1.--Seasonal distribution of 247 cases of swine stillbirths, Nagano Prefecture, Japan, 1951[1]

Swine stillbirths	Month of occurrence												Total
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Number of cases.....	1	2	1	6	2	2	0	12	73	102	46	0	247
Occurrence rate, percent.....	ND[1]	ND	ND	ND	ND	ND	0	4.3	13.6	17.0	12.2	0	([2])

[1] Adapted from Akiyama and others (1).
[2] ND = not determined.

Table 2.--Breeding data of 247 cases of sows which delivered stillborn fetuses, Nagano Prefecture, Japan, 1951[1]

Gestation No.	Month of breeding												Total
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
1st.....	2	1	1	2	49	76	43	6	2	1	2	4	189
2nd.....	--	--	--	2	5	13	14	1	--	--	--	1	36
3rd.....	--	--	--	--	2	6	4	--	--	--	--	1	13
4th, 5th.....	--	--	--	--	3	1	4	1	--	--	--	--	9
Total.....	2	1	1	4	59	96	65	8	2	1	2	6	247

[1] Adapted from Akujama and others (1).

Table 3.--Farrowing data of 247 cases of dams that delivered abnormal fetuses, Nagano Prefecture, Japan, 1951)

Gestation	Dams	Normal offspring		Abnormal offspring		Normal and abnormal offsprings	
		Total	Pigs per sow average	Total	Pigs per sow average	Total	Pigs per sow average
	Number	Number	Number	Number	Number	Number	Number
First.....	189	352	1.9	1,282	6.8	1,634	8.6
Second.....	36	126	3.5	176	4.9	302	8.4
Third.....	13	49	3.8	71	5.5	120	9.2
Fourth, fifth.....	9	64	7.1	58	6.4	122	13.5
Total.....	247	591	2.4	1,587	6.4	2,178	8.8

Farrowing data of those dams that produced abnormal litters are shown in figure 4 and table 3. In approximately 60 percent of the cases there were no living pigs at the end of gestation. The remainder farrowed various numbers of both normal and abnormal pigs. Progeny of these affected dams varied in size and character even in the same litter. These abnormalities include small mummified fetuses, near term dead fetuses and living pigs. Many pigs died in a few hours after birth showing symptoms of congenital tremor or convulsion. Some of these piglets or stillborn fetuses produced morphologic lesions of the central nervous system, such as hydrocephalus, cerebellar hypolasia, and spinal hypomyelinogenesis.

When the central nervous system was examined histopathologically, nonpurulent encephalomyelitis lesions were recognized in many cases (17, 35, 42). The main lesions of these cases consisted of degeneration of nervous cells, proliferation of glial cells, glial nodules, and perivascular cuffing (35).

An agent identified as Japanese encephalitis virus was first isolated by Matumoto and others (17) from the brains of three piglets of typical cases that occurred near the end of July 1948. Subsequently, a number of strains of Japanese encephalitis virus have been isolated from stillborn fetuses or congenitally infected piglets (5, 10, 12, 30, 37, 38, 42).

High incidence of stillbirths also occurred among swine imported from foreign countries, such as Australia, North American, and Europe. According to the serological surveys, these imported animals were free from Japanese encephalitis at the time of arrival, but possessed antibodies to JEV following the summer incidence of the disease (3).

Table 4 shows the results observed by Chikatsune and others (3). These animals were imported from the United States in winter, and raised at a livestock breeding center in Japan. They had been bred between May 6 and June 18, before the epidemics of Japanese encephalitis,

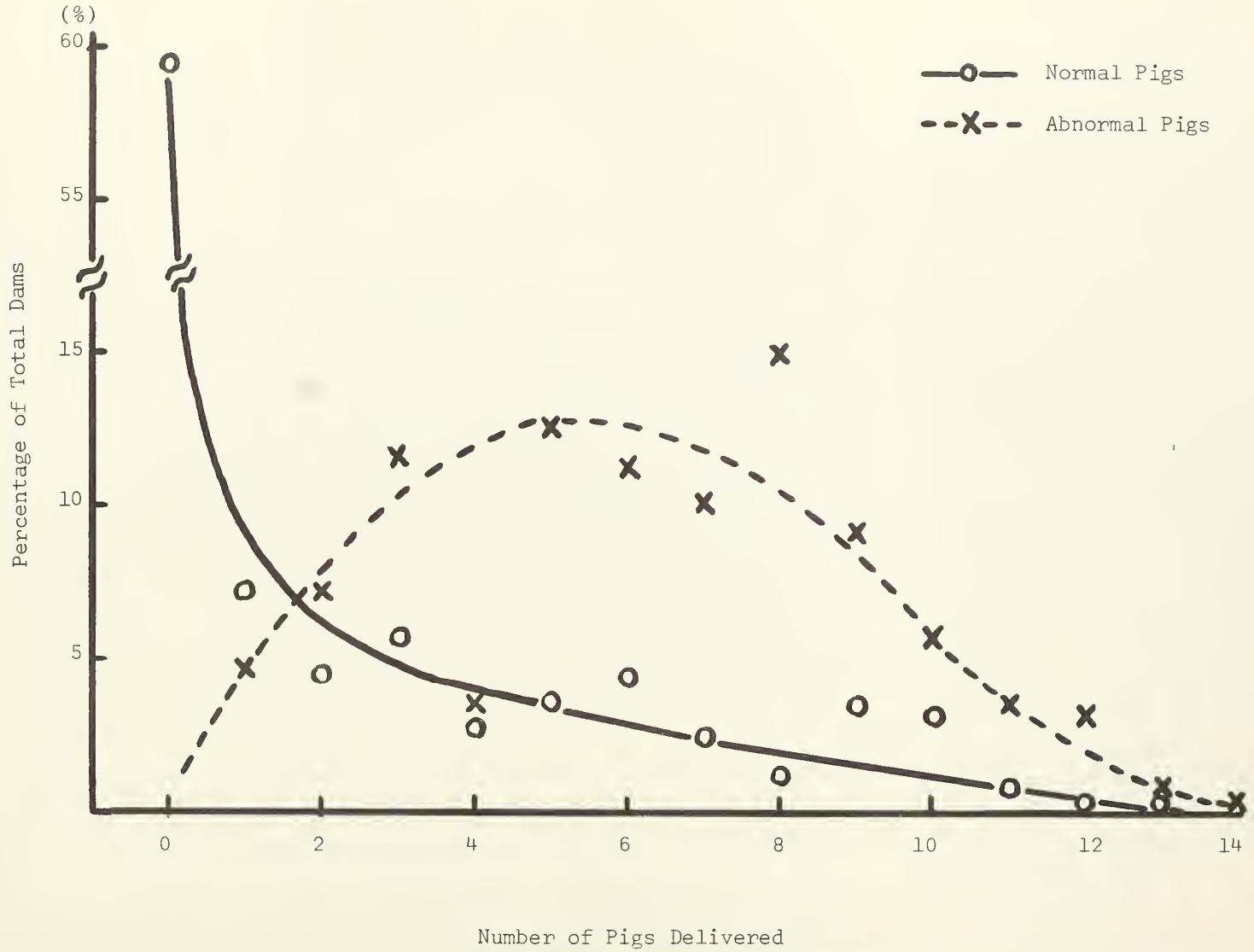

Figure 4.--Farrowing data of 247 cases of dams that delivered abnormal fetuses in Nagano Prefecture, in 1951. (Adapted from Akiyama and others, 1.)

Table 4.--Farrowing data of sows imported from the United States[1]

Sow no.	Date of breeding	Date of farrowing	Number of farrowed			Total
			Normal	Mummified	Stillborn	
1	5/6	8/28	6	5	3	14
2	5/12	9/3	9	0	0	9
3	5/13	9/4	3	0	3	6
4	5/12	9/3	4	0	0	4
5	5/12	9/3	3	0	3	6
6	5/12	9/7	2	6	2	10
7	5/30	9/21	0	5	4	9
8	5/30	9/21	0	3	5	8
9	5/25	9/16	0	5	5	10
10	6/9	10/2	0	3	1	4
11	6/18	10/9	8	0	2	10
Total			35	27	28	90
Average			3.2	2.5	2.5	8.2

[1] Adapted from Chitkatsune and others (3).

and had farrowed during August 28 and October 9. After farrowing, serum samples were collected and tested for the presence of antibodies to JEV. As the result, all were positive to complement-fixation, hemagglutination-inhibition, and neutralization to JEV. These results indicated that they had been infected with JEV during pregnancy. It is also suggested that occurrence of stillbirths among imported swine might be caused by the infection of Japanese encephalitis virus. However, no attempt was made to recover the causative agent from those cases.

Experimental Infection

Piglets

The susceptibility of piglets to Japanese encephalitis virus was studied by several investigators (18, 21, 29, 31, 33).[5,6] For their experiments, most investigators used natural maternal nursing piglets purchased from Hokkaido, where Japanese encephalitis is known to be nonepidemic. Recently, however, colostrum-deprived pathogen-free piglets either delivered naturally or derived by hysterectomy were also used by some investigators.[6,7]

Different strains of Japanese excephalitis virus from affected human brain, horse brain, swine blood, and brain of stillborn swine fetus were studied for their pathogenicity in piglets. There were no fundamental difference in clinical and virological aspects between conventional and specific pathogen-free piglets, following inoculation of the virus originated from different sources.

Clinical response in piglets inoculated by the intracerebral route was characterized by elevation of body temperature, manifestation of the typical encephalitis symptoms, and death within a few days after onset of disease. In piglets inoculated intravenously, intranasally, intradermally, or subcutaneously, the temperature response appeared, but the pigs survived without showing signs of nervous symptoms, except for a few animals, which received the virus intravenously or intranasally. These manifested symptoms of a fatal encephalitis.

[5] Nakamura, J., Nakamura, H., and Nozaki, I. Experimental infection of piglets and lambs with Japanese encephalitis virus. Twelveth annual meeting of the Japanese Society for Virologist. 1964.

[6] Sazawa, H., Sugimori, T., Morimoto, T., and Miura, Y. Unpublished data. 1966.

[7] Kodama, K., and Sasaki, F. Experimental infection of colostrum-deprived piglets with Japanese encephalitis virus. Sixty-first meeting of the Japanese Society of Veterinary Science. 1966.

Table 5.--Distribution of the virus in colostrum-deprived, pathogen-free piglets inoculated with the Fuji strain of Japanese encephalitis virus

Piglet No.	2	6	7
Route of inoculation[1]	S.C.	I.C.	I.N.
Day Postinfection	3	3	3
(Specimen)			
Cerebral Cortex	[2] --	[3] 5.0	--
Occipital lobe	--	5.2	5.7
Thalamus	--	5.5	5.7
Brain stem	--	4.8	2.2
Spinal cord (cervical)	--	3.2	--
Spinal cord (lumbar)	--	1.8	--
Lymph node (cervical)	4.3	--	5.3
Lymph node (prescapular)	3.5	3.7	--
Lymph node (mesenteric)	4.3	4.7	3.6
Lung	2.2	2.7	1.7
Liver	2.0	1.7	1.7
Spleen	4.3	4.5	4.3
Kidney	2.3	2.2	1.7
Blood	--	3.0	0.8

[1] S.C. = subcutaneously, I.C. = intracerebral, I.N. = intranasal.
[2] Negative when tested 10^{-1} diluted of tissue emulsion or undiluted heparinized blood.
[3] Infected titer (log TCID 50/g.).

Viremia appeared in almost all piglets inoculated with the virus by any route mentioned above. It could be detected as early as 24 hours after infection and lasted 2 to 5 days.

The distribution of the virus in various body tissues of piglets were examined by several investigators (31).[8] Table 5 shows one of the results of the virus titration carried out on necropsy specimens of colostrum-deprived pathogen-free piglets inoculated with the Fuji strain of Japanese encephalitis virus.[9] Virus could be recovered from various tissues of piglets. High titers were obtained from tissues of central nervous system of piglets inoculated intracerebrally or intranasally. Comparatively higher titers of virus were demonstrated in lymph nodes and spleen of all animals regardless of the route of infection.

Neutralizing or hemagglutination-inhibition antibodies were demonstrated in all surviving animals. They were first detected on the 7th day postinfection and reached high titers by the 14th to 21st day.

Histopathological findings of artifically infected piglets were reported by Mochizuk and others (19). The specimens used for their pathological examination were the same as those selected in the experiments of Shimizu and Kawakami (31).

The main lesions of the piglets inoculated with the virus intracerebrally were degeneration of nervous cells, neuronophagia, proliferation of glial cells, perivascular cuffing and petechial hemorrhage. These lesions were distributed diffusely throughout the nervous system, including thalamus, substantia nigra, nucleus basalis, cerebral cortex, cerebellum, and the anterior horns of the spinal cord. The gray matter was more affected than the white matter. Severe leptomeningitis was also noticed in the brain but somewhat less in the spinal cord. The germinal center of follicles in lymph nodes were swollen and filled with many proliferated lymph cells. Fatty degeneration and infiltration of lymphoid cells were also observed in liver and kidney. Essentially, similar lesions were observed in the central nervous system of piglets inoculated intravenously, but the degree of the changes were slight as compared with those mentioned above.

[8] See footnotes 6 and 7.
[9] See footnote 6.

These findings indicated that piglets were quite susceptible to infection with Japanese encephalitis virus, particularly to intracerebral inoculation, which produced fatal encephalitis. Viremia was observed in almost all piglets regardless of the route of infection, and antibodies were produced in all surviving animals.

Pregnant Swine

The published work on experimental infection of pregnant swine with Japanese encephalitis virus was limited. Shimizu and his associates (32) confirmed Japanese encephalitis virus as the etiological agent of swine stillbirth observed in Japan during the summer months.

Table 6 shows the summarized results of their experiments. In the first experiment, five pregnant swine were inoculated intravenously with Fuji strain which was isolated from the brain of a stillborn fetus (30) and had undergone a number of mouse brain passages.

Swine Nos. 1 and 2 were sacrificed 8 and 16 days, respectively, after inoculation and the remaining three dams (Nos. 3, 4, and 5), were observed until natural farrowing. All the fetuses or newborn piglets obtained from infected dams were alive and normal in size except two fetuses, from each of swine Nos. 1 and 3. They were small in size and mummified. It was observed that these two mummified fetuses could not have been caused by the virus inoculation, when one considered the size of fetuses and the time of the virus inoculation.

Some of the normal fetuses and newborn piglets were sacrified immediately after hysterectomy or natural farrowing, and emulsions of various organs were tested for the presence of the virus with negative results.

These results indicated that the Fuji strain of Japanese encephalitis virus completely failed to infect fetuses and to produce stillbirth by inoculation into the pregnant swine.

In the second experiment, six pregnant swine were inoculated with the Kanagawa strain, which was isolated from the fetal case of bovine encephalitis (34) and passaged through mouse brain only four or five times. Four of the six swine were killed at short intervals after inoculation, and the remaining two were observed until the natural farrowing or sacrificed near the end of gestation.

Table 6.--Summarized results of experimental infection with Japanese encephalitis virus in pregnant sows[1]

Exp. No.	Sow No.	Inoculum			Days after copulation when inoculated	Days after inoculation when delivered or slaughtered[2]	Results			Virus recovery from fetus[3]
		Strain	No. of passages in mouse brain	Doses mouse LD_{50}			Normal	Abnormal		
								Mummified	Hydrocephalus	
I.....	1	Fuji	34	$10^{9.5}$	69	S 8	5	1	0	-
	2		39	$10^{9.3}$	92	S16	9	0	0	-
	3		90-100	$10^{6.5}$	36	D77	5	0	0	-
	4		90-100	$10^{6.5}$	46	D61	14	0	0	-
	5		90-100	$10^{6.5}$	97	D17	5	1	0	-
II.....	6	Kanagawa	4	$10^{7.5}$	40	D84	0	10	0	ND
	7		4	$10^{8.5}$	49	S 7	11	0	0	+
	8		4	$10^{7.2}$	81	S10	12	0	0	-
	9		5	$10^{6.0}$	43	S10	9	1	0	+
	10		5	$10^{6.0}$	46	S22	13	1	0	+
	11		5	$10^{6.0}$	46	S62	0	4	3	-

[1] Adapted from Shimizu and others (32).
[2] S or D indicated that dam was slaughtered and delivered, respectively.
[3] ND = Not done.

The latter two pregnant swine, one (No. 6) delivered 10 small mummified fetuses and another (No. 11) gave three live fetuses with hydrocephalus and subcutaneous edema and four dead mummified fetuses. These findings compared favorably with the results found in natural cases of typical stillbirth.

Four dams, which were killed at short intervals following inoculation, had normal fetuses except two litters containing one mummified fetus each. These mummified fetuses were probably caused by nonspecific death. In swine No. 8, which received the virus at later stage of gestation, all of 12 fetuses had no virus; whereas, a number of fetuses from swine Nos. 7, 9, and 10 that were inoculated at earlier stage of gestation yielded the virus.

The detailed data of virus distribution in the fetuses are shown in table 7. As can be seen in the table, the virus distribution in fetuses was not uniform. Some fetuses contained no virus in the organs tested or only a small amount of virus in a few organs; from others, the virus was readily recovered from all organs examined. In addition, the virus could be recovered from placenta in some fetuses.

Viremia was demonstrated in all pregnant swine, following inoculation of the virus intravenously. The virus was easily recovered from the blood samples immediately after inoculation, but became undemonstrable shortly after inoculation. Thereafter the virus reappeared when tested one day after inoculation, then decreased gradually until the 4th day (table 8). This suggested that the virus in the blood stream gets into fetuses probably through placenta by some unknown mechanism. The recovery of the virus from placental tissues gives a support for this hypothesis. The duration of viremia was longer in swine inoculated with Kanagawa strain than those of inoculated with Fuji strain.

Neutralizing antibodies became positive 1 week after inoculation, their maximum was reached after 2 weeks. Thereafter insignificant changes were observed during the test periods.

In the first experiment the Fuji strain was used and no stillbirth was observed. However, the Kanagawa strain, which was used in the second experiment, produced typical stillbirth. The Fuji strain had undergone a number of mouse brain passages when used for the experiment, but the Kanagawa strain was only at the 4th or 5th passage level. It is highly possible that this difference of the results in producing the stillbirth might be due to the difference in pathogenicity of those two strains.

Table 7.--Distribution of the virus in fetuses of the dams inoculated intravenously with the Kanagawa strain of Japanese encephalitis virus[1]

Sow No.	Days after copulation when inoc.	Days after inoc. when slaughtered	Fetus No.	Organ Tested[2]							
				Brain	Spinal cord	Blood	Lung	Liver	Spleen	Kidney	Placenta
9	43	10	1	-	-	-	-	-	-	-	-
			2	-	-	-	-	-	-	-	+
			3	+	+	+	+	+	+	+	+
			4	-	-	-	-	-	-	-	-
			5	-	-	-	+	-	+	-	+
			6	not done (mummified fetus)							
			7	-	-	-	-	-	-	-	-
			8	-	-	-	-	-	-	-	-
			9	-	-	-	+	-	-	-	-
			10	+	-	-	-	-	-	-	-

[1] Adapted from Shimizu and others (32).
[2] 10 percent tissue suspensions or undiluted blood were used for mouse inoculation + Virus recovery positive, - Virus recovery negative.

Table 8.--Viremia in pregnant sows inoculated intravenously with Japanese encephalitis virus[1]

Exp. No.	Sow No.	Inoculum	Hours after inoculation		Days after inoculation[2]						
			0	6	1	2	3	4	5	6	7
I	1	Fuji	+	-	+	+	-	-	-	-	-
	2		+	-	+	-	-	-	-	-	-
	3		+	-	+	+	-	-	-	-	-
	4		+	-	+	+	-	-	-	-	-
	5		+	-	+	-	-	-	-	-	-
II	6	Kanagawa	+	-	+	+	+	+	-	-	-
	7		+	-	+	+	+	+	-	-	-
	8		+	-	+	+	+	+	-	-	-
	9		+	-	-	ND	+	ND	-	ND	-

[1] Adapted from Shimizu and others (32).
[2] ND = not done.

Summary

Epizootic swine stillbirth has occurred in Japan in the late summer and early fall for many years. The seasonal outbreak was correlated to the epidemic of Japanese excephalitis among humans and horses and to the time of occurrence of Japanese excephalitis virus infection among swine.

A number of strains of Japanese encephalitis virus have been isolated from the typical cases of stillborn fetuses and congenitally affected piglets. Histopathological findings of such stillborn fetuses revealed nonpurulent encephalitis lesions.

The virus of Japanese encephalitis produced stillborn fetuses experimentally comparative to those observed in natural cases when virus was inoculated intravenously into swine during an early stage of pregnancy.

These epizootiological and ethiological findings make clear that the virus of Japanese encephalitis is a causative agent of this disease in swine.

ADDENDUM

Experimental Swine Stillbirth Produced by Hemagglutinating Virus of Japan (HVJ)

A virus called hemagglutinating virus of Japan (HVJ), or Sendai virus, classified recently as myxovirus parainfluenza 1, was isolated from human (15, 44), swine (28, 43), and mouse (4) sources in Japan since 1952.

Table 9.--Experimental infection of pregnant sows with hemagglutinating virus of Japan (HVJ)[1]

Sow No.	Days After copulation when inoculated	Inoculum Doses[2]		Clinical symptoms		Antibody response[3]		Number Farrowed			Number of live pigs surviving 5 days[4]	Virus recovery[5]
		Route	Milli-liter	Fever	Cough	Pre-	Post-	Live	Mummi-fied	Still-born		
129	39	i.n.	10	+	-	<8	256	3	7	0	0	+ (MF)
127	43	i.n.	20	-	+	<8	512	5	5	4	0	+ (SF)
[6] 285	61	i.n.	1	-	+	<8	128	4	0	0	ND	-
126	89	i.n.	1	-	-	<8	64	6	0	0	4	+ (DP)
132	90	i.n.	10	+	+	<8	128	9	0	0	5	-
134	102	i.n.	20	-	+	<8	64	3	0	1	3	+ (SF)
133	61	Control		-	-	<8	<8	6	0	0	6	
128	69	Control		-	-	<8	<8	7	0	0	7	
135	85	Control		-	-	<8	<8	4	0	0	3	

[1] Adapted from Sasahara and others (27).
[2] Undiluted infectious allantoic fluids of Tanashi strain, isolated from a pig in embryonating hen's eggs, passaged 3 times in allantoic cavity, were used for inoculation; i.n. = intranasally.
[3] Hemagglutination-inhibition antibody. Figure shows reciprocal dilution of serum.
[4] ND = not determined.
[5] MF = mummified fetus, SF = stillborn fetus, DP = dead piglet.
[6] This sow was sacrificed 27 days after inoculation.

This virus was pathogenic for swine, especially for young piglets, producing clinical and pathological changes of bronchopneumonitis when inoculated intranasally (26). A widespread distribution of neutralizing antibodies to the HVJ were observed among swine in Japan (27).

Three cases of stillbirths were produced experimentally by Sasahara and others (27), by inoculating the virus intranasally into the pregnant swine as shown in table 9. The virus used for inoculation was the newly isolated Tanashi strain which had been passaged three times in allantoic cavity of embryonating hen's eggs since its isolation from a pig (26).

Clinical signs of respiratory disorder were observed in some infected animals and all developed HI antibody to the HVJ after inoculation. Of six inoculated animals, one sow (No. 129), which received the virus 39 days after breeding, delivered seven mummified fetuses. Another sow (No. 127), inoculated with the virus 43 days after breeding, gave birth to five mummified fetuses and four stillborn fetuses. The remaining four sows that received the virus at a late stage of gestation delivered normal living pigs, except for one sow (No. 134) that produced one stillborn fetus.

Hydrocephalus or cerebromalacia which was noted in experimental cases of stillborn fetuses produced by Japanese encephalitis virus (32) was not observed in these cases of stillborn fetuses. Histopathologically, two of four stillborn fetuses had a slight amount of hemorrhage in the Virchow-Robin spaces without inflammatory or necrotic changes.

The virus was recovered from one mummified fetus to No. 129, two stillborn fetuses of No. 127, one stillborn fetus of No. 134, and one piglet of No. 126 that had died within 24 hours after birth.

It was clear that the virus of hemagglutinating virus of Japan had a capability of producing stillbirth in pregnant swine, but at the present time, its importance in field outbreaks of stillbirth among swine in Japan is unknown.

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SMEDI VIRUSES[1]

By H. W. Dunne[2]

The SMEDI viruses were first recognized as pathogenic agents with an affinity for embryonic tissue in 1963, when an agent, cytopathic for porcine kidney cells, was isolated from a dead fetus (6). The fetus had died in utero a very few days before hysterectomy at 75 days of gestation. The uterus also contained seven live pigs, one other dead pig, and three mummies. Since then more than 12 virus strains were isolated from fetuses or stillborn pigs in the same laboratory.

The name SMEDI was derived from the first letter of each of the conditions produced by the effect of the viruses in utero which included stillbirth, mummification, embryonic death, and infertility. Although abortions were reported in isolated cases, they were not definitely proved to be associated with the viruses named above.

Classification of SMEDI Viruses

The SMEDI isolates were picorna viruses. They were resistant to heating at 56° C. for 2 hours. They were also resistant to 20 percent ether for 24 hours at 4°. They passed 500 Å filters and appeared to be 300 Å in diameter according to electron microscope studies

They were hemagglutination negative (bovine, procine, ovine, avian, and human O erythrocytes) and hemadsorption negative. They were examined bacteriologically and the results were negative for both bacteria and PPLO before inoculations into pregnant gilts. Staining with acridine organge using the method of Schiffner indicated that the virus stimulated the production of ribonucleic acid with cultured cells. These characteristics placed the agents into the category of the picorna viruses of swine (5, 14, 18).

After a serologic comparison with more than 50 local isolates and more than 20 strains isolated by others (table 1), the SMEDI viruses were placed into four serologic groups, SMEDI A, B, C, and D of P1, P6, P7 and P8, respectively. If some of the weak relationships were considered, D could be considered a subgroup of A. This may be resolved after more strains become available. Currently there appeared to be 15 North American porcine picorna types (7). Two of these, P6 and P7 or SMEDI B and A, respectively, represent 65 percent of all viruses isolated at the Pennsylvania State University laboratories (7). These two groups also appeared to have rather wide distribution, possibly even worldwide. As table 1 indicates, Group P6 (SMEDI B) was found in Ohio, Canada, and England (3, 4, 7, 8, 13).[3]

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[3] Cartwright, Sheila F. Personal communication, May 1967.

Table 1.--Serologic relationships of procine enterovirus type strains as identified by various authorities

Suggested N.A. Type	U. S. A.				Canada	United Kingdom	Japan
	DUNNE	BOHL	KASZA	BANKOWSKI	GRIEG	BETTS HARDING	MORIMOTO
P 1	PS34 (SMEDI C)	ECPO 9		E 1		Talfan	J1 (SF12)
2		ECPO 3					
3			03b				
4	PS36						
5				E 4		T 80	J2 (SFK10)
6	PS12 PS14 (SMEDI B)	ECPO 6	02b		PE 1	F 34	
7	PS27 (SMEDI A)	ECPO 5	01i			V 13A (Cartwright)	J4 (SF16)
8	PS32 (SMEDI D)					V 13?	
9			04b				
10		ECPO 2	05i				
11				CHICO			
12		ECPO 1					
13	PS37						
14	PS38						J7 (G-D)
15		ECPO4					

——— Suggested North American Type strains.

Group P7 (SMEDI A) was found to cross react with swine picorna viruses from Ohio, England, and Japan (4, 7, 13, 14, 16).[4] [5] A third group, P1 (SMEDI C) was Talfan-Teschen related (7).[4] Although the number of Pennsylvania isolates in this group was not great, related or identical viruses included not only Talfan (England) and Teschen (England) but also isolates from Japan, Ohio, and California (4, 7, 10, 16, 19).[4] A fourth group P8 (SMEDI D) was comprised of only two isolates. These two had no complete cross serum neutralization with any of the other 70 strains. They were, however, neutralized by the serum of three strains of group P7 (SMEDI A), but the antibodies of the SMEDI D group did not neutralize any of the other strains suggesting a possible sub-group reaction. A similar impression was gained by Cartwright of England[4], who suggested subgrouping of the P7 (SMEDI A) V13 group. (7, 15).[4] This group was characterized serologically by a wide range of irregular neutralizations within the group.

Hog cholera was eliminated by pig inoculation and challenge tests and with fluorescent antibody reactions. Pseudorabies was negated by the subcutaneous inoculation of rabbits.

Epidemiologic Observations

In a detailed study of herd cases (6), evidence of infection with SMEDI viruses appeared to manifest itself first by the presence of an abnormal number of stillbirths and mummified fetuses. Later, a few too many breeding animals appeared to be barren and repeat breeding was common. Generally, there had been no previous history of the disease, or it appeared to recur only at periodic intervals. Of the affected sows, none had suffered a similar affliction before. In one case it appeared that only first litter gilts were involved. On another farm, in the first year of

[4] See footnote 3.
[5] Morimoto, T. Personal communication, June 1967.

raising pigs, both sows with previous successful litters, and first litter gilts were involved. On one farm where the condition appeared to be endemic, incidence appeared to be greatest about every 2-1/2 to 3 years.

Histories on other farms with a similar condition but from which no viruses were recovered paralleled this observation. On a farm where this did not seem to be the case, two viruses (SMEDI A and B) were isolated from fetuses several months apart, suggesting possible alternate involvement with two or more SMEDI viruses, or other viral agents. On one farm where a continuous farrowing operation (four sows farrowing every 2 months) was being practiced, every 6 weeks to 2 months, new sows were added to a pasture holding bred sows. Trouble began 2 months after the addition of a newly purchased bred sow that farrowed one live and one mummified pig. At that time, farrowings were characterized only by pigs being born dead. Later, mummified fetuses, as many as 11 per litter were observed. Where pigs were full size but stillborn, there appeared to be a sequential problem of repeat breeding. The disease affected bred sows over a period of 7 months from August to March. Thereafter all farrowings again were normal. In herds where repeat breeding did not appear to be a problem with sows that had farrowed abnormal litters, subsequent conception was followed by the birth of large, normal litters without complications.

The mode of transmission appeared to be either oral or respiratory. The random positions of dead fetuses in the uterus and the ages at which they had died suggested that the virus in field cases must have entered the uterus through the bloodstream. These observations minimized the possibility of veneral transmission.

Experimentally Infected, First Litter, SPF Gilts

Experimental infection of first litter gilts was accomplished by injecting subcutaneously cell-cultured virus (SMEDI A or B) 21 to 27 days after breeding. All gilts were primary specific-pathogen-free pigs (SPF), but a few irregularities developed when such animals were maintained in outside isolation lots. It appeared that on at least two occasions, during the wintertime when starlings were present in great numbers, at least two control gilts were exposed, developed antibodies, and had poor litters. Also, at least two gilts developed antibodies between the time they were tested for susceptibility and the time of experimental exposure. Gilts so exposed were resistant to the experimentally injected viruses and had normal litters. Notwithstanding, these exceptions and including them in the total data, the results of several experiments (successful and unsuccessful) were combined to produce table 2. As can be determined from the table, 64 gilts were utilized in all experiments; 28 principals were nonimmune; seven were immune, and 29 were nonimmune controls. Hysterectomies were performed on all gilts unless they farrowed early, that is, before 112 days. Placentae were obtained from such animals but no examinations were made of the uteri or ovaries. Data were accumulated at hysterectomy on the number of nongravid gilts that had not been observed to return to estrus after the first 21-day interval following breeding. Also, the number of live, stillborn, and mummified fetuses taken from the uterus at termination of pregnancy were recorded. In later experiments, it became obvious that by counting the number of corpus luteum and subtracting from this figure the total number of

Table 2.--SMEDI virus exposure of gilts 21 to 27 days pregnant

Group	Number of gilts	Percent nongravid	Average number of pigs[1]			Percent 5-day living	Excess C.L.[2] average
			Live	Stillborn	Mummified		
Nonimmune.....	28	10.1	6.2	0.14	2.4	[3] 27.2	[4] 4.6
Immune.....	7	0	9.6	0	.14	[5] 84.0	[6] 1.3
All controls.....	29	6.8	10.4	.04	.37	[7] 73.1	[8] 2.37

[1] Nongravid gilts included in computing averages. [2] C.L. = corpus luteum. [3] 8 litters. [4] 9 litters. [5] 2 litters. [6] 3 litters. [7] 8 litters. [8] 17 litters.

live, stillborn, and mummified fetuses one could determine the number of ova and embryos which survived less than 30 days and were absorbed. This was recorded as "excess CL." Likewise, the ability of the newborn to survive without colostrum could be compared best by determining survival on a given date. Five days were arbitrarily selected. In later experiments, these data were accumulated and accurately tabulated.

The table shows that the number of nongravid gilts was greatest in the nonimmune principals and lowest (actually none) in the group immune to the viruses used. The average number of live pigs was only 6.2 per litter for the exposed gilts, 10.4 for the controls, and 8.5 for the immune group. The number of stillborn were not significant in any of the three groups but were slightly higher in the nonimmune principals and none in the immune group. Mummified fetuses (figs. 1, 2, and 3) were six times greater in number in the virus exposed, nonimmune principals than in

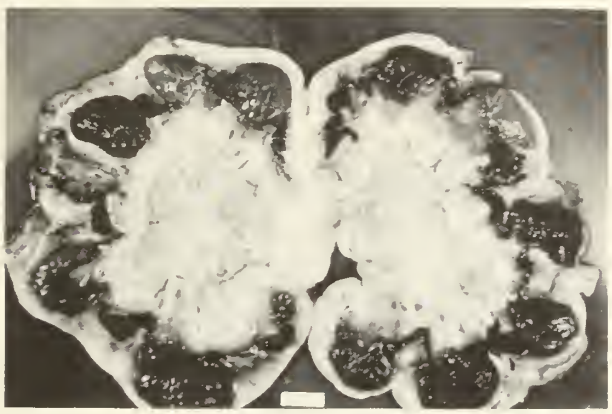

Figure 1.--The uterus from gilt 75 with only mummified fetuses at end of gestation. The gilt was infected with virus SMEDI A at 25th day following breeding. There was no correlation between position and size of fetuses in the uterine horn. All fetuses appeared to be in the 40th to 55th days of gestation. Such sows do not farrow or return to heat.

Figure 2.--Joined, mummified fetuses from gilt 68 following inoculation with virus SMEDI A on 25th day after conception. Four of the five fetuses in the order shown above were in one horn of the uterus. The fetus in the center was in the other uterine horn with four live pigs. The largest pig in the above group was near term in size.

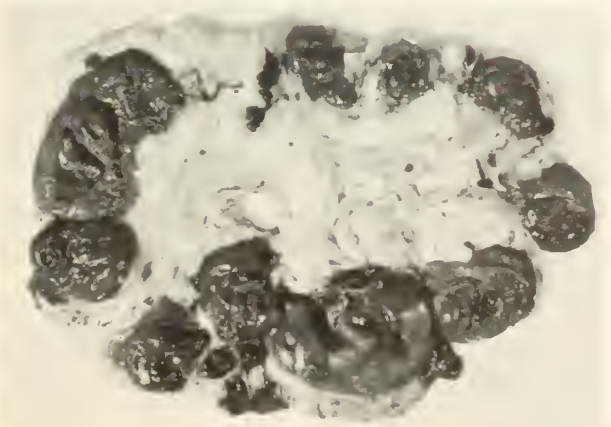

Figure 3.--Eleven mummified fetuses ranging in gestation age from 40 to 70 days were found at term in the uterus of gilt 06. The gilt had been infected with virus SMEDI B on 25th day after breeding. Notice the tendency in one horn of the uterus toward progressive death in terms of age of the fetus. Near the cervix, the fetuses were probably less than 40 days old, and the fetuses were increasingly older to the largest (near 70 days) at the ovarian end of the uterine horn.

the controls, and 15 times as great as in the immune group. Of particular interest was the apparent effect of virus infection upon survival of the colostrum-deprived pig even though virus could not be detected, except rarely, in pigs dying after birth. The 5-day survival data show that only 27.2 percent of colostrum deprived pigs born alive to eight nonimmune, virus exposed gilts survived for 5 days, whereas, 73.1 percent of pigs born to 29 control gilts survived the same period. Although the number of immune gilts (two) was not significant, 84 percent of the two litters survived 5 days. About twice as many ova (4.6) failed to develop into fetuses or died as embryos and were absorbed in the virus exposed, nonimmune group as in the control group (2.37). Again the immune group contained only three gilts but the ova that failed in this group were only one-third (1.3) that of the nonimmune, virus exposed principals.

Occasionally both in field cases and experimentally in virus exposed susceptible gilts, all of the fetuses in the uterus would die between 35 and 50 days of gestation (figs. 1 and 3). Such fetuses were not absorbed. If the gilt were allowed to go past term she did not farrow. There was no abortion and no return to estrus. The fetuses acted as foreign bodies but were not expelled. Such gilts were sold as "nonbreeders" when the condition developed in a farm herd.

Other Clinical Signs and Pathological Changes

Exposed gilts were not noticed to react clinically to the inoculations of the virus. Handling of the pregnant animals was kept to a minimum to avoid undue stress as another variable. Therefore, blood samples for cell counts, virus isolations, and antibody levels were not taken from the gilts in these earlier experiments.

Pigs dying shortly after birth or born dead had few lesions. Apart from mummification, the occasional atresia ani, cleft palate, and mild edema there were few gross lesions that could be detected. Histopathologically, lesions were very mild. When they could be detected, they included perivascular cuffing (fig. 4), edema, and endothelial degeneration in the cerebrum, medulla and, to a lesser extent, in the cerebellum.

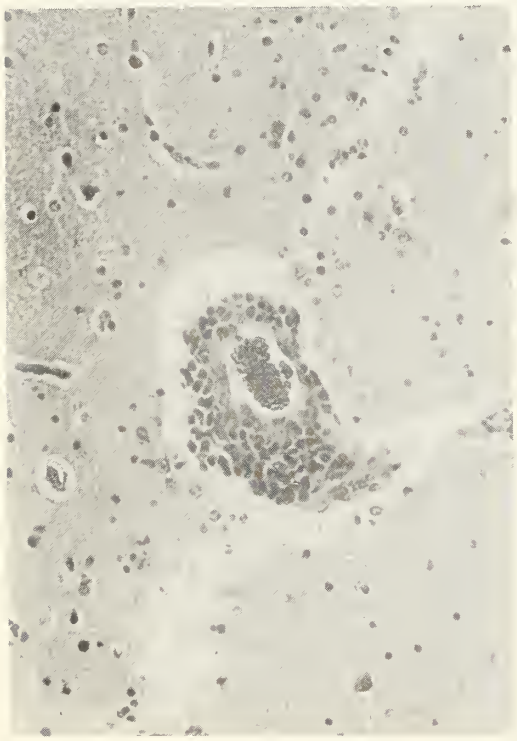

Figure 4.--Perivascular cuffing and edema of the cerebrum of a pig dying within 10 minutes after being removed, by hysterectomy, from an infected sow. Notice the pericellular edema. X 80. H & F stain.

Focal microgliosis was found occasionally in the cerebrum. Intercellular and intracellular edema was observed throughout the brain in a few cases. Pigs dying at 6 days or more after birth had more lesions of bacterial infection than of virus infection.

Summary

SMEDI viruses A, B, C, and D were isolated from stillborn pigs or fetuses. They were picorna viruses and were capable of causing the death of embryos with absorption, the death of fetuses with mummification or stillbirth, and the death of newborn within a few days after birth. Transmission appeared to be other than venereal, and immunity appeared to be effective against recurrence of the reproductive problem. The viruses were shown to be widespread, occurring in several of the States and in Canada, Europe, and Asia.

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DISCUSSION

Dr. Kernkamp:

Before you sit down, Dr. Dunne, I want you to tell me what this SMEDI is again.

Dr. Dunne:

It is stillbirth, mummification, embryonic death, and infertility.

Dr. Carbrey:

Did you get that classification of viruses in Wilmer's book?

Dr. Dunne:

As far as the task of classification is concerned, Cartwright really is doing most of the work on this right now. He has been doing a lot of work with these viruses. The difficulty is, of course, that we want to classify the viruses, and there are certain problems involved in importing the viruses. We thank the USDA for the opportunity to get these viruses into the country. We have been importing these for something like 2 1/2 to 3 years now. USDA has been working very hard to give us some help; I am not condemning them, but it is a real tough nut to crack. We just decided to start with our own classifications. This is where we are.

Dr. Carbrey:

We have isolated several entero viruses from swine, usually from pigs older than the group under observation here. We have the same problem of classifying them.

Dr. Dunne:

I would be very glad to send you all of our specimens.

III. SUMMARY OF SYMPOSIUM

By R. E. Omohundro[1]

This afternoon I am scheduled to present a paper summarizing what has gone on at this meeting. Frankly, after two or three papers, it was obvious that I could not properly summarize the technical papers presented here. Therefore, I asked Howard Dunne to request a paragraph or two from each speaker, to be used as the summary of the meeting.

Many have been received for which I am most grateful. With regard to the few remaining, we have summarized the comments as we understood them. If by chance these summaries are misinterpretations or place undue emphasis on certain points, I apologize.

It is a great pleasure to witness the development of science in the swine field. The physiologist, the geneticist, the nutritionist, the immunologist, the endocrinologist, the virologist, the bacteriologist and the parasitologist in the swine field are all to be congratulated. Please accept my thanks for the opportunity to be here and to learn.

Sincerely, I bring you greetings from the Director's Office. We join our allied organizations in hoping this meeting has been worthwhile and productive to each of you.

The purpose of the Symposium was to bring together those who are actively engaged with the various problems concerning the intrauterine environment of swine so that both the participants and the audience could develop a meaningful exchange of information.

The first session was devoted to environmental, management, nutritional, and physiological factors. In this session the need for standard nomenclature of some of the basic terms was pointed out. For example, we need mutually accepted definitions of such terms as: embryo, fetus, stillbirth, and SPF.

Practically 40 percent of all ova are lost before parturition. Embryonic losses occur largely during three periods--that is, the first 25 days; the 60th to 69th day; and the last 21 days of pregnancy. The effect environmental temperature has on embryonic losses was noted. While temperature may affect the ovulation rate, the dam may die of heat prostration before embryonic mortality and abortions occur.

The SPF program in Nebraska was discussed. The program is built around a group of standards designed to maintain herds free of specific pathogens. If the herd is not free of these specific agents, there are provisions to disqualify it. SPF should not be used synonymously with such terms as germ-free inasmuch as SPF denotes that the herd is free of the specified disease agents.

High energy feeding before breeding produces greater ovulation rates in the gilt, but if continued into early gestation results in increased embryonic death, both in the gilt and the sow. Fertilization rate is unaffected. Evidence from embryo transfer studies indicates that an unfavorable uterine environment rather than a defect in the gamete is responsible.

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The gilt and sow can tolerate very low levels of protein during gestation without affecting embryonic and fetal mortality. However, the feeding of too low a protein intake before breeding will reduce ovulation rate.

When supplying reduced levels of feeding to the gilt and sow, the diet should be fortified with vitamins and minerals, so that the daily requirements of the pregnant sow are met.

The age of both the ova and the sperm affect embryo development. Consequently, there is an optimal time for ova fertilization, and this time does not cover the entire estrus period of the sow. The effect of intrauterine crowding or ova development was examined, as well as, the opposite. In the latter instance, the sow, in some manner, "counts" her embryos and "decides" whether the pregnancy should continue.

With regard to genetics, total wastage of ova before births may range between 30 and 35 percent. All cannot be classed as embryonic death losses, and some of these could be due to genetic factors. Chromosomal abnormalities can cause death losses, the example cited being a boar that sired litters one half the size of litters from the same dams but sired by different boars. Imcompatability between the fetus and the dam results in fetal death loss. Inbreeding multiplies these factors.

The newborn pig is relatively immunologically incompetent. This incompetence seems to be a consequence of its deficiency in natural antibody. The quantity of natural antibody needed for immunologic competence appears to be small, and the level of competence can be significantly increased by small amounts of antibody. This deficiency in natural antibody is normally corrected by passive acquisition of maternal antibody from the colostrum. The level of natural antibody will also increase in the pig by synthesis. In this way the pig will acquire complete immunologic competence in a few weeks.

Leptospirosis is a factor causing abortion in swine. Chemically killed vaccine prevents abortions but does not halt renal infection nor development of carrier swine. Dihydrostreptomycin is effective in treatment of leptospirosis and it is affective against the renal form.

Reproductive failure due to swine brucellosis in the United States is caused by Brucellosis suis type 1 or type 3. There is no evidence that embryonic death or fetal anatomic abnormalities result from B. suis in pregnant swine. Abortion is the most serious complication in female swine, occurring almost any time during the gestation period, although the majority of sows affected with brucellosis do not abort. Stillbirth or birth of weak pigs occurs infrequently. Sterility can result from chronic endometritis, usually as a sequela of abortion caused by B. suis.

Abortion may be caused by miscellaneous organisms. Many organisms have been associated with sporadic abortion and it is likely that still others will be found. Those most commonly involved, include Streptococcus sp., Escherichia coli, Corynebacterium pyogenes, C. suis, Staphylococcus aureus, Erysipelas rhuseopathiae, and Mycobacterium tuberculosis. These organisms have been named as causative factors when present in profuse, pure cultures. A difficulty arises when cultures are not isolated under sterile conditions. Then one does not know whether they are contaminants, synergists secondary invaders or primary agents.

Fungi, particularly Aspergillus fumigatus can cause abortion. Consideration also should be given to the aflatoxin producing fungi and to Giberella zeas.

The pathology of myoclonia congenita was described with emphasis on brain lesions involving leucocytic infiltration and endothelial proliferation. Various causes were suggested including hereditary factors, neurotropic viruses, hog cholera virus, and others. It was suggested that myoclonia congenita might be a disease common to different species, being manifested as a nervous disturbance in baby pigs, but affecting other species in a different manner.

Various parasites cause fetal loss. Strongyloidal infections in the fetus appear to develop to the larval stage becoming patent 2 to 4 days following birth. The triggering mechanism is not known.

In addition, colostral transmission may occur. Stephanuris dentatus causes fetal loss. Earlier, investigators thought that the period required for patent infections to develop was long. However, later information indicates this period may be much shorter. Ascarides infection was

discussed. The liver damage in baby pigs was thought to be more the result of sensitization rather than to larval migration.

SMEDI viruses, A, B, C, and D were isolated from stillborn pigs or fetuses. They were picorna viruses and were capable of causing the death of embryos with absorption, the death of newborn within a few days after birth. Transmission appeared to be other than venerial, and immunity appeared to be effective against recurrence of the reproductive problem. The viruses were shown to be widespread, occurring in several States, Canada, Europe, and Asia.

Infections of susceptible gilts at 24 days of gestation with attenuated hog cholera virus resulted in an increased number of barren degenerated infected embryos.

Infection of susceptible gilts at 60 days of gestation resulted in increased numbers of mummies, stillborns, and pigs dying before five days of age. Hog cholera virus was recovered from all litters in the 60-day group.

Virus recovered from affected pigs was lethal to 4-week-old pigs and to four of six, 8-week-old pigs, but had only an immunizing effect on 5-month-old pigs.

Carrier or chronic pigs developed in two of the 8-week-old inoculated pigs. Upon stressing, they broke with classical hog cholera.

In utero transmission did not occur in 12 immune sows given virulent hog cholera virus. On the other hand, transmission occurred in 80 and 100 percent of natural farrowed litters of susceptible sows given attenuated vaccine and a field strain of low virulence, respectively. Confirmation of in utero transmission was obtained in all but one case by detection of hog cholera virus by the fluorescent antibody, tissue culture technique (FATCT).

Vaccination of susceptible sows resulted in high mortality of baby pigs; approximately half were killed in utero by the virus. The majority of the live pigs were weak and died within 8 days. Mortality in mature swine, abortions, and early fetal arrest with death in utero resulted when susceptible sows were exposed to a strain of low virulence. Foremost, was death in utero of the baby pigs, approximately 65 percent were dead at birth.

Laboratory findings were compared with clinical histories on 64 herds with baby pig losses, abortions, and fetal abnormalities. Hog cholera virus was isolated from 18 of these herds. When the field diagnosis was uncomplicated myoclonia congenital, hog cholera virus was isolated from only one of 17 herds.

Hog cholera virus strains from 11 of these herds were characterized by inoculation into susceptible pigs. Five strains were avirulent, three were of low virulence, and three of high virulence. One of the low virulent strains produced a chronic course of disease in the pig lasting 93 days. The pig had periods of normal clinical appearance during this time, although virulent hog cholera virus could be recovered from its blood and contact swine consistently succumbed to fatal infection with hog cholera.

Thirteen sows at various stages of pregnancy were exposed to the "S" strain of pseudorabies virus. One died 7 days after exposure. One aborted at 7 days post-exposure, was subsequently rebred, and farrowed a litter of pigs which were devoid of neutralizing antibody at weaning. Five sows were killed within a day or two of farrowing. The uteri contained live and dead fetuses. There were more dead fetuses, 10 per 12 and 9 per 12, in uteri of sows exposed at 45 days of pregnancy than at later times to 93 days. Virus was isolated from three tissues among two embryos in the uterus of one sow. This indicates that the virus does cross to the embryo in the uterus. Six other sows were permitted to farrow following exposure. One farrowed 14 macerated embryos 21 days later than the expected farrowing date. Five sows were permitted to farrow and the living pigs were tested for neutralizing antibodies in their serum after weaning and all were negative. These were found to be susceptible by sampling of some. Some were tested for antibodies before receiving colostrum and none were found. Three of the five have been rebred and one has farrowed without incident.

The following observations have been made as a result of these experiments:

1. Pregnant sows intranasally exposed to pseudorabies virus may die, abort the fetuses, carry macerated fetuses beyond anticipated farrowing dates, or carry varying ratios of live to dead fetuses to term.

2. Fetal anomalies were not found among fetuses carried by sows exposed to pseudorabies virus during pregnancy. They were born live and normal or dead.

3. Fetuses died in the uterus in random arrangement and at various times during the pregnancy indicating an interesting pattern of response to virus present in the sow.

4. Sows that abort farrowed normal litters on subsequent breeding and pregnancy.

5. Efforts to observe evidence of virus carriers among pigs born live or among the sows have been made and no evidence was found.

Considering the various disciplines represented here to examine a single subject reminds me of the ancient parable of the six blind men who were looking at the elephant. This is not to say we are blind--but each of us is liable to look at the problem area with certain restrictions. As you will recall, each of the blind men described the elephant differently. One thought it much like a snake. Another felt it consisted of four pillars. And still another called it a rope. And so, they wrangled on far into the night--each insisting that he alone was right.

Let us hope that we can learn from this example--that we can take this opportunity to view "the elephant" from more than one angle. For unlike the six we are not blind--and hopefully not deaf.

Gentlemen, during the past 2 days we have had an opportunity to take a good look at a goodly part of that elephant! Thank you.

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